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Coupling Effect of Phosphorus with Organic Manures and Bioinoculant on Growth and Yield of Black Gram (*Vigna mungo* (L.) Hepper) in Black Calcareous Soil

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***Abstract*:** To evaluate the effect of phosphorus in conjoint with organic manures and bioinoculant for optimizing the phosphorus availability in calcareous soil, a field experiment was carried out in Kalligudi block of Madurai district using black gram (VBN 8) as a test crop. Twelve treatments were formulated with different combinations of vermicompost, farmyard manure, *Bacillus megaterium* (phosphorus solubilizing bacteria) and phosphorus fertilizer. Among the different combinations, application of 100% of P_2O_5 on soil test crop response (STCR) basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} with a recommended dose of nitrogen and potassium fertilizer recorded the maximum plant height (43.85 cm), root length (23.82 cm), nodules $plant^{-1}$ (27.42), dry matter production (2349 kg ha^{-1}), number of pods $plant^{-1}$ (28.52), seeds pod^{-1} (6.98), 100 seed weight (4.49 g), grain yield (936 kg ha^{-1}) and haulm yield (1472 kg ha^{-1}) at harvest stage. The lowest growth and yield attributes were recorded in absolute control. Application of organic manures and bioinoculant along with inorganic fertilizer in black calcareous soil led to a greater yield of black gram of 35.9% than the application of inorganic fertilizer alone. The study concluded that the application of phosphorus fertilizer along with organic manures and phosphorus solubilizing bacteria (*Bacillus megaterium*) in calcareous soils improves the phosphorus use efficiency and ultimately it will enhance the growth and yield of black gram.

***Keywords*:** Phosphorus, Calcareous soil, *Bacillus megaterium*, Vermicompost

Black gram (*Vigna mungo* (L.) Hepper) is one of the most highly prized pulse crops occupying a unique position in Indian agriculture. India is the leading producer and consumer of black gram in the world. About 70% of the world's black gram production is grown in India. In 2020-21, India produces around 24.5 lakh tonnes of black gram from 4.6 million hectares of area with average productivity of 533 kg ha^{-1} (Black gram outlook 2021). Nearly 19% of India's total pulse acreage is used to grow black gram, which makes up 23% of India's total pulse production. Various environmental factors such as light, water, temperature, and nutrient availability have a significant impact on the growth and development of black gram. Phosphorus deficiency in soil has a negative influence on legume production because it is necessary for energy transformation in nodules, root growth and development and enhanced nitrogen fixation (Udvardi and Poole 2013), photosynthesis, nucleic acid synthesis, resistance to soil-borne root diseases and seed production (Akram et al 2017). As a result, P-deficient soil and limited availability of phosphorus impose significant constraints on the productivity of crops (Zhang et al 2014).

The majority of pulses in India are cultivated in low fertile and problematic soils. In general pulse crops mostly prefer neutral soil reactions and are extremely sensitive to acidic, saline, and alkaline soil conditions (Singh et al 2013). In the background of agricultural problem soils, calcareous soils generally contain high amount of calcium carbonate content which has a significant impact on soil properties related to crop growth. Most soil nutrients, such as N, P, K, S, Zn, Fe, Mn, Cu, and B are often less available to plants due to the high pH and $CaCO_3$ content in calcareous soil (FAO, 2020). The $CaCO_3$ in calcareous soil reacts with native and applied phosphorus and forms the calcium phosphate compounds of lower solubility ($CaCO_3 + 2PO_4^{3-} \rightarrow Ca_3(PO_4)_2 + 3CO_2\uparrow$). Application of phosphorus fertilizers at normal rates and with conventional methods in calcareous soil may not give optimal crop yield and quality. Improving crop production in calcareous soil requires increasing phosphorus use efficiency through appropriate P management measures. The conjoint application of organic manures and bioinoculant along with inorganic phosphorus fertilizer was found to increase the efficiency of applied phosphorus fertilizer in calcareous soil. Phosphate solubilizing bacteria and organic manures application are promising strategies to increase the bioavailability of insoluble soil phosphorus for plant use by producing organic acids, thereby increasing P solubility (Alori et al 2017). Keeping foregoing in mind, a field experiment was carried out to investigate the potential of using organic

manures and phosphorus solubilizing bacteria (PSB) in conjunction with phosphorus fertilizer to improve black gram growth and yield in calcareous soil.

MATERIAL AND METHODS

Experimental site: A field trial was conducted in a farmer's field in Kalligudi block (9.65° N 77.92° E) of Madurai district, Tamil Nadu state during 2021-22. The experimental site received a mean annual rainfall of 840 mm and mean minimum and maximum temperature of 21.6°C and 39°C. The experimental site belongs to the Peelamedu soil series. According to USDA soil taxonomy, it has been classified as "Fine clayey montmorillonitic isohyperthermic *Typic Chromustert*".

Treatment details: The experimental field was laid out with an individual plot size of 20 m^2 (5×4 m) in a randomized block design with three replications. VBN 8 black gram was used as a test crop. Twelve treatments were followed with a combination of farmyard manure, vermicompost, *Bacillus megaterium* and chemical fertilizer (Table 1). Except for T_1, T_2, T_3 and T_8, all the treatments received soil test crop response-based N and K_2O as basal. Urea, single super phosphate (SSP) and muriate of potash (MOP) were used as a source of nitrogen, phosphorus and potassium, respectively.

Preparation of phosphorus fertilizer in conjoint with organic manures: To make enriched phosphorus fertilizer, two different sources of organic manures *viz.,* vermicompost and farmyard manure were taken and mixed with phosphorus fertilizer in different ratios *viz.,* 100% of P_2O_5 on STCR basis mixed with farmyard manure (1:10 ratio), 75% of P_2O_5 on STCR basis mixed with farmyard manure (1:10 ratio), 100% of P_2O_5 on STCR basis mixed with vermicompost (1:5 ratio) and 75% of P_2O_5 on STCR basis mixed with vermicompost (1:5 ratio). These combinations were prepared and kept for incubation at 60% moisture content in grow bag over a period of one month.

Table 1. Treatment details

Treatment	Details
T_1	Absolute control
T_2	Recommended N, P_2O_5, K_2O @ 25:50:25 kg ha^{-1}
T_3	100% of P_2O_5 on STCR basis alone
T_4	100% of P_2O_5 on STCR basis incubated for 30 days with farm yard manure at 1:10 ratio
T_5	75% of P_2O_5 on STCR basis incubated for 30 days with farm yard manure at 1:10 ratio
T_6	100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio
T_7	75% of P_2O_5 on STCR basis incubated with vermicompost for 30 days at 1:5 ratio
T_8	100% of P_2O_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}

Data collection and analysis: For initial soil characterization, soil samples were collected from the field at 0 to 15 cm depth. The collected soil samples were shade dried and sieved through a 2 mm sieve. To estimate organic carbon content, the samples were sieved through a 0.2 mm sieve. The processed samples were analyzed for texture (International pipette method, Piper, 1966), bulk density, particle density, pore space (Core sampler method, Gupta and Dakshinamoorthy,1980), pH (Potentiometry, Jackson 1973), electrical conductivity (Conductometry, Jackson 1973), cation exchange capacity (CEC) (Neutral normal ammonium acetate method, Jackson 1973), anion exchange capacity (AEC) (Magnesium sulphate – Barium chloride method, Gillman, 1979), organic carbon (Dichromate wet digestion method, Walkley and Black, 1934), free $CaCO_3$ (Rapid titration method, Piper 1966), available nitrogen (Alkaline permanganate method, Subbaiah and Asija 1956), available phosphorus (Olsen method, Olsen, 1954), available potassium (Neutral normal NH_4OAc method, Stanford and English 1949), and DTPA extractable micronutrients Zn, Mn, Fe and Cu (Atomic Absorption Spectrophotometer, Lindsay and Norvell 1978). The initial soil properties are given in Table 2. The dataset collected was statistically analyzed with the AGRES software package.

Growth Attributes

Plant height: Co-application of phosphorus fertilizer with organic manures and bioinoculant significantly influenced the plant height at different growth stages of black gram (Table 3). The plant height ranged from 12.08 to 21.51, 17.30 to 35.01 and 20.52 to 43.85 cm at vegetative, flowering and harvest stages, respectively. The tallest plant was recorded in the treatment supplied with 100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} at vegetative, flowering and harvest stage which was followed with the treatment received 75% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} at vegetative, flowering and harvest. The shortest plant of 12.08, 17.30 and 20.52 cm at vegetative, flowering and harvest stage, respectively was found with absolute control. The synergistic effect of phosphorus, vermicompost and PSB application might have enhanced the root activity and root nodulation of plants, resulting in higher uptake of plant nutrients in adequate quantities and increased plant height. This line was in

Table 2. Initial soil properties

Particulars	Value
Mechanical composition	
Clay (%)	34.76
Silt (%)	20.41
Fine sand (%)	17.01
Coarse sand (%)	25.68
Texture class	Sandy clay loam
Physical properties	
Bulk density (Mg m^{-3})	1.34
Particle density (Mg m^{-3})	2.50
Porosity (%)	41
Physico-chemical properties	
pH	8.32
EC (dSm^{-1})	0.18
CEC (c mol (p^+) kg^{-1})	25.62
AEC (c mol (p^-) kg^{-1})	2.34
Chemical properties	
Total Sesquioxide (%)	5.32
Free $CaCO_3$ (%)	7.63
Organic Carbon (g kg^{-1})	3.8
Alkaline $KMnO_4$ - N (kg ha^{-1})	234
Olsen - P (kg ha^{-1})	10.34
NH_4OAc - K (kg ha^{-1})	214.24
$CaCl_2$ - Sulphur (ppm)	13.92
Exchangeable Calcium (c mol (p^+) kg^{-1})	13.97
Exchangeable Magnesium (c mol (p^+) kg^{-1})	6.42
DTPA extractable - Fe (mg kg^{-1})	2.33
DTPA extractable - Mn (mg kg^{-1})	7.37
DTPA extractable - Zn (mg kg^{-1})	1.24
DTPA extractable - Cu (mg kg^{-1})	2.54

accordance with the findings of Kuntyastuti and Sutrisno (2017) and Hu et al (2014). Patel et al (2020) also reported that phosphorus promotes the new cell formation and elongation of cells, increases plant vigour and hastens leaf development, which aids in the harvesting of more solar energy and the better utilization of nitrogen, resulting in increased plant growth.

Root length: The phosphorus fertilization along with organic manures and bioinoculant significantly influenced the root length at different growth stages of black gram (Table 3). Phosphorus fertilization along with organic manures and bioinoculant upgraded the root length and maximum root length of 14.58, 19.37 and 23.82 cm was observed with the treatment received 100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} at vegetative, flowering and harvest stage, respectively. The least root length of 6.79, 10.84 and 11.32 cm was with absolute control at vegetative, flowering and harvest stages, respectively. Vermicompost and PSB combination results in a higher root length this could be due to the addition of inorganic P alone is converted into a number of reaction products in soil, the bulk of which were low soluble orthophosphates. When combined with vermicompost and PSB, the fixed P is dislodged resulting in higher phosphorus bioavailability (Naik and Mehera 2022), which in turn increases the root length of black gram. Yadav et al (2019) reported that, the application of vermicompost with bioinoculant and inorganic fertilizer plays a critical part in root development and proliferation, which leads to improved nodule formation and nitrogen fixation by supplying the assimilates to the roots as well as a better rhizosphere environment for root growth and development. The addition of vermicompost might have improved the aeration,

Table 3. Effect of phosphorus in conjoint with organic manures and bioinoculant on plant height and root length of black gram

Treatments	Plant height (cm)			Root length (cm)		
	Vegetative stage	Flowering stage	At harvest	Vegetative stage	Flowering stage	At harvest
T_1	12.08	17.30	20.52	6.79	10.84	11.32
T_2	14.79	21.71	27.98	9.78	14.03	16.20
T_3	13.35	18.21	22.08	7.31	11.51	12.07
T_4	16.68	26.06	32.89	11.41	15.75	18.43
T_5	15.76	24.10	30.93	10.60	14.96	17.31
T_6	18.42	28.46	35.50	12.17	16.65	19.81
T_7	17.39	26.53	33.57	11.46	15.92	18.69
T_8	13.78	19.41	23.96	8.75	12.34	14.29
T_9	20.30	32.76	40.16	13.62	18.45	22.36
T_{10}	19.41	30.71	37.99	12.88	17.68	21.20
T_{11}	21.51	35.01	43.85	14.58	19.37	23.82
T_{12}	20.69	33.25	40.90	13.69	18.52	22.73
CD (p=0.05)	0.73	0.85	1.54	0.50	0.65	0.69

drainage, and created a desirable soil environment for deeper root penetration and better nutrient extraction from the soil.

Root nodules: The application of inorganic phosphorus fertilizer along with organic manures and bioinoculant had a positive effect on nodule count at the flowering stage of black gram (Table 4). Among the various treatments, the root nodule count of 27.42 was found to be superior in the treatment received 100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} at the flowering stage which was followed by the addition of 75% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} (25.28). The lowest nodule count of 7.39 was recorded in absolute control. The relatively higher phosphorus availability in the rhizosphere region might have aided the *Rhizobium* activity which resulted in a greater number of nodules plant^{-1} (Kumar and Yadav 2018). This might be the reason for a higher number of nodules in the treatment received phosphorus than in absolute control. Rekha et al (2018) reported that the increase in the number of nodules plant^{-1} could be attributed to more favourable conditions for microorganisms or the formation of a conducive soil for microbe proliferation by the application of organic manures with inorganic fertilizers. Better nodulation in PSB inoculated treatment might be due to increased P availability through PSB, which enhances root nodule count and biological nitrogen fixation (Nadeem et al 2018).

Dry matter production of black gram: The integrated application of phosphorus fertilizer along with organic manures and bioinoculant had a significant effect on the dry matter production of black gram (Table 4). The dry matter production was extended from 630 to 1235, 951 to 1855 and 1200 to 2349 kg ha^{-1} at vegetative, flowering and harvest stages, respectively. Among the treatments, 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}) recorded significantly a higher dry matter production of 1235, 1855 and 2349 kg ha^{-1} at vegetative, flowering and harvest stage, respectively and this was followed by T_{12}. The lowest dry matter production was noted in control (T_1). The highest dry matter accumulation in the above-mentioned treatment might be due to the combined application of organic manures with phosphorus fertilizer to the crop which increased the availability of nutrients by promoting early root growth, resulting in increased nutrient absorption from deeper layers of soil and ultimately enhanced the vegetative growth of plants. A similar result was also reported by Banotra et al (2021). Increased phosphorus availability might have increased nodulation activity, cell development, plant vigour, and vegetative growth, which might be one of the reasons for the highest dry matter production in the treatment received phosphorus fertilizer than absolute control. These results are in agreement with those of Chena et al (2017) and Bekele et al (2019).

Yield Attributes of Black Gram

Number of pods plant^{-1} and number grains pod^{-1}: The number of pods plant^{-1} and grains pod^{-1} were found statistically significant due to the application of various combinations of organic manure, bioinoculant and

Table 4. Effect of phosphorus in conjoint with organic manures and bioinoculant on nodules plant^{-1} and dry matter production (kg ha^{-1}) of black gram

Treatments	Number of nodules plant^{-1}	Dry matter production (kg ha^{-1})		
	Flowering stage	Vegetative stage	Flowering stage	At harvest
T_1	7.39	630	951	1200
T_2	14.39	822	1207	1539
T_3	8.26	669	1006	1264
T_4	18.25	947	1401	1768
T_5	16.46	889	1318	1670
T_6	20.42	1028	1532	1920
T_7	18.72	972	1446	1817
T_8	11.76	722	1077	1376
T_9	25.11	1154	1717	2176
T_{10}	22.31	1099	1634	2071
T_{11}	27.42	1235	1855	2349
T_{12}	25.28	1177	1758	2228
CD (p=0.05)	0.83	36.29	54.66	62.42

phosphorus fertilizer over control (Table 5). 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} produced the maximum number of pods and grains $plant^{-1}$ of 28.52 & 6.98 respectively. Treatment which received RDF alone produced a comparatively lower number of pods and grains $plant^{-1}$ of 16.73 & 4.68 respectively. This might be due to the use of inorganic fertilizers increased the availability of essential nutrients to crop plants, whereas the use of organic manures, notably vermicompost, increased the activity of microorganisms in soils, which improves the nutrient solubility and availability to the plants. As a result, the combined effect of organic and inorganic nutrient sources improved the yield attributes of black gram. Sonnet et al (2020) reported that phosphorus fertilization stimulates the plant for flowering and fruiting which leads to the development of more pods $plant^{-1}$. Application of phosphorus resulted in increased carbohydrate accumulation and their remobilization to reproductive parts of the plant, being the closest sink resulted in increased flowering, fruiting and seed formation (Shamsurahman et al 2020). This might be the reason for a higher pods $plant^{-1}$ in phosphorus fertilized plots than in absolute control.

Test weight: The phosphorus fertilization along with organic manures and bioinoculant significantly influenced the test weight of black gram (Table 5). The maximum test weight of 4.49 g was observed in the treatment 100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} (T_{11}) closely followed by T_{12}. The least test weight of 3.80 g was recorded in absolute control. This might be due to the reason that, vermicompost is an excellent source of N, P, K, and organic acids which are released during the decomposition of vermicompost and played an important role in improved nutrient availability in both early and later stages of crop growth. Sharma et al (2021) stated that phosphorus fertilization had increased the plant photosynthesis activity and aided in the development of a more extensive root system, allowing the plant to extract more water and nutrients from higher soil depth, which resulted in improved plant growth and yield attributes.

Grain yield: The positive response was observed with the application of phosphorus fertilizer in conjoint with organic manures and bioinoculant as compared to the application of inorganic P fertilizer alone (Table 5). The maximum grain yield of 936 kg ha^{-1} was registered with T_{11}. The integration of vermicompost and bioinoculant along with phosphorus fertilizer has improved the black gram yield by 35.89% over the use of a recommended dose of fertilizer alone. The minimum grain yield of 432 kg ha^{-1} was recorded in absolute control (T_1). The highest grain yield of the treatments received organic manures and bioinoculant might be due to the ability of PSB and organic manures to secrete organic acids for solubilization of precipitated phosphorus (calcium phosphate) and lowering the pH of the surrounding bulk and rhizosphere soil which creates a favourable environment for higher phosphorus availability and grain yield. This finding is in agreement with the findings of Wahid et al (2020). Singh et al (2018) reported that the increased translocation might have occurred as a result of increased potassium and phosphorus uptake, which are responsible for the rapid and easy translocation of photosynthates from source to sink which in turn increased the grain yield. PSB has been found to produce

Table 5. Effect of phosphorus in conjoint with organic manures and bioinoculant on yield attributes of black gram

Treatments	Number of pods $plant^{-1}$	Number of grains pod^{-1}	Test weight (g)	Grain yield (kg ha^{-1})	Haulm yield (kg ha^{-1})
T_1	8.90	4.01	3.80	432	882
T_2	16.73	4.68	3.99	600	1065
T_3	9.78	4.19	3.85	456	926
T_4	21.04	5.23	4.14	728	1185
T_5	19.37	4.97	4.09	682	1131
T_6	22.91	5.62	4.23	781	1268
T_7	21.22	5.36	4.16	739	1210
T_8	11.14	4.41	3.91	497	975
T_9	26.75	6.18	4.41	880	1383
T_{10}	25.16	5.92	4.35	837	1326
T_{11}	28.52	6.98	4.49	936	1472
T_{12}	26.92	6.23	4.43	891	1414
CD (p=0.05)	0.83	0.17	0.04	24.25	42.04

vitamins (Vitamin A, B_1, B_2, B_3, C and E), Indole Acetic Acid (IAA), Naphthalene acetic acid and gibberellin-like compounds. These growth factors, combined with improved nutritional conditions might have played a significant role in increasing grain yield (Sharma et al 2021). This might be the reason for a higher grain yield in the treatments inoculated with PSB than in non-inoculated treatments.

Haulm yield: The application of various treatments had a significant effect on haulm yield of black gram and it extended from 882 to 1472 kg ha^{-1} (Table 5). Among the treatments, 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}) registered significantly the highest haulm yield of 1472 kg ha^{-1} and this was followed by T_{12} with the value of 1414 kg ha^{-1}. The lowest haulm yield was noted in absolute control (882 kg ha^{-1}). An increased in haulm yield of 27.64% was recorded by 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} over recommended dose of fertilizer alone. This might be due to the production of growth-promoting substances and organic acids during organic manures decomposition might have facilitated easy availability and absorption of macro and micronutrients. This might be the reason for higher haulm yield in the treatment received organic manures, bioinoculant and inorganic P fertilizer than the application of inorganic phosphorus fertilizer alone. Similar finding was also reported by Khan et al (2017).

CONCLUSION

Application of phosphorus in conjoint with bioinoculant and organic's sources significantly enhanced the growth and yield attributes of black gram in calcareous soil. 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} with nitrogen and potassium fertilizer in calcareous soil significantly increases the growth and yield of black gram. Application of organic manures with PSB and chemical fertilizers reduces the phosphorus fixation and also solubilizes the fixed phosphorus in calcareous soil as calcium phosphate by the production of various organics acid which leads to a higher phosphorus availability, growth and yield of crops.

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