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RESEARCH ARTICLE

Synergistic effects of biochar and biopriming on growth and yield in blackgram (*Vigna mungo*) var. VBN 11

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Abstract

Blackgram (*Vigna mungo*) is a significant pulse crop, widely cultivated for its nutritional value and role in improving soil fertility through biological nitrogen fixation. However, its production is constrained by poor seed vigour, low nutrient use efficiency and vulnerability to abiotic and biotic stresses, which limit its yield potential. Biochar, a novel, low-cost and renewable carbonaceous material, has gained attention due to its numerous functional groups and tunable mesoporous structure, which contribute to enhanced soil fertility, nutrient retention and act as a carrier for the microbial inoculum. Similarly, biopriming, an eco-friendly seed treatment method, has emerged as a promising strategy to enhance seed germination, vigour and resilience under stress conditions. To explore the synergistic benefits of biochar and biopriming, an experiment was conducted at laboratory and field levels to assess their combined effect on blackgram (*Vigna mungo*) var. VBN 11. Seeds treated with Rhizobium 10 % + biochar at 100 g/kg recorded significantly higher physiological, biochemical, growth and yield parameters compared to untreated seeds, which exhibited lower performance across all traits. These findings highlight the potential of integrating biochar coating and biopriming to overcome production constraints in blackgram by improving seed quality, enhancing nutrient uptake and promoting sustainable crop productivity.

Keywords: biochar; biofertilizer; blackgram; production; quality

Introduction

Legumes are widely grown around the world and their nutritional and economic value is widely recognised. They are the only high-protein component of the average Indian diet, with over 10 million tonnes consumed annually (1). Blackgram is the third important legume crop in India. Blackgram area accounts for about 15.7 % of India's total pulse acreage and contributes 9.09 % of total pulse production. Despite its significance, blackgram productivity is constrained by challenges such as poor seedling vigour, nutrient deficiencies and abiotic stresses, necessitating innovative approaches to enhance its growth and yield.

Biofertilizers are organic products comprising living cells of various microorganisms capable of converting nutritionally important elements from unavailable to available form via biological processes. Inoculation of these biofertilizers to crops was found to increase the crop yield by about 10 %-15 % under on-farm conditions (2). These efficient strains of biofertilizer were used for the treatment of soil and seed. Seed treatment with biofertilizer, mainly biopriming, was a promising technology to increase the seedling quality. Biopriming with *Rhizobium*, phosphobacteria (*Bacillus megaterium*) and Potash Releasing Bacteria (KRB) supplies the essential nutrients like nitrogen, phosphorus and potassium to the developing seeds.

Early seedling growth is a critical requirement for crop survival and productivity. Biochar treatment can vastly improve such early seedling development (3). Global climate change, in the form of rising temperatures and changed soil moisture, is expected to reduce food crop yields during the next 50 years (4). Global changes mostly involve temperature rise and water scarcity. The absence of moisture in the soil has a direct impact on reduced seed germination and seedling growth in plants. Since biochar withholds moisture, the current experiment was designed to explore the seed germination and yield in blackgram by biopriming and biochar seed coating.

Materials and Methods

Blackgram variety VBN 11 seeds were collected from the Agricultural Research Station, Paramakudi. The bioinoculants, viz. *Rhizobium*, *Bacillus megaterium* (Phosphobacteria), KRB (*Peanibacillus mucilaginosus*) were obtained from the Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai. Biochar made from green waste of the *Prosopis* plant was obtained from the Department of Soil Science and Analytical Chemistry, Agricultural College and Research Institute, Madurai. The blackgram seeds were bioprimed with the best concentration of bioinoculants, i.e.

Rhizobium 10 %, Phosphobacteria 10 % and KRB 3 %. Then the seeds were shade-dried to their original moisture content. The dried seeds were coated with biochar at 100 g per kg of seed using starch (1 %) as adhesive. The treatments were T_0 – Untreated seeds (control), T_1 – Hydropriming + Biochar at 100 g/kg of seed T_2- *Bacillus* 10 % + Biochar at 100 g/kg of seed, T_3 - *Rhizobium* 10 % + Biochar at 100 g/kg of seed, T_4 -KRB 3 % + Biochar at 100 g/kg of seed, T_5- *Bacillus* 10 % + KRB 3 % + Biochar at 100 g/kg of seed, T_6- *Bacillus* 10 % + *Rhizobium* 10 % + Biochar at 100 g/kg of seed, T_7- *Rhizobium* 10 % + KRB 3 % + Biochar at 100 g/kg of seed, T_8 - *Bacillus* 10 % + *Rhizobium* 10 % + KRB 3 % + Biochar at 100 g/kg of seed. The germination test was conducted as per the ISTA procedure and evaluated for different quality parameters, viz. germination percentage (%), shoot length (cm), root length (cm), dry matter production per 10 seedlings (g) and vigour index values. During the germination test, the speed of germination was also evaluated (5). The electrical conductivity was measured by soaking 25 seeds in 50 mL of water for 3 hr. Dehydrogenase activity, Catalase activity and Peroxidase activity were also assessed by using standard procedures (6-8). Total microbial population was estimated by using the standard method and expressed as CFU × 10^6/g (9). During field performance of bioprimed and biochar-coated seeds, the parameters like leaf area index, dry weight (g), chlorophyll content, crop growth rate (g/m^2/d), number of nodules and pods and plant yield (g) were analysed.

Statistical analysis

The data collected were statistically analysed using AGRES software (10). Whenever necessary, the percentage data were changed using the arcsine transformation and the critical difference at the 5 % level was determined.

Results and Discussion

Biochar was used as a seed coating material since significant yield increases occur when appropriate biochar formulations are applied at an ideal application rate to accommodate site-specific restrictions, nutrient and water limitations (11). Among the treatments, T_3 (*Rhizobium* 10 % + biochar coating 100 g/kg) recorded the highest speed of germination (16.83) over the control (9.35) (Table 1). The accelerated germination of primed seeds was due to an increased rate of cell division (12). Biochar application leads to early seedling development due to sorption of the allelochemicals. T_6 (*Rhizobium* 10 % + *Bacillus* 10 % + biochar coating 100 g/kg) also performed well next to T_3 with an increase in speed of germination (15.81) (13). The combined application of biofertilizers increases the hormone gibberellin that triggers the α-amylase activity and other germination-related enzymes like nuclease and protease that are involved in hydrolysis and assimilation of starch (14).

The higher germination percentage was observed in T_3 (98 %), while the lower germination percentage was recorded in the control (80 %). Similar result of increasing germination due to the inoculation of *Rhizobium* was studied in Bengal gram, greengram, rice and greengram (15-17). The germination was also enhanced by biochar due to its carbon-based nature, high water-holding capacity and the presence of nutrients such as N, P, K, Ca, Mg, Cu and Fe. This increase in germination was noticed in previous studies (19). Regarding seedling length, the highest root length was recorded in T_3 (10.35 cm) and the lowest was recorded in control (9.15 cm) (Fig. 1). The maximum shoot length of 12.89 cm was observed in T_3 while the minimum value (10.23 cm) was registered in control (T_0). The reason may be due to the fertilising impact of biochar resulted in nutrient release from damaged or degraded storage organ tissue via hydrolysis, as documented in sorghum (20). Application of rice husk biochar considerably enhanced the shoot biomass of rice and tomato (21).The increase in shoot length was observed since microorganisms promote seedling growth by producing metabolites similar to plant hormones such as abscisic acid, gibberellins, indole acetic acid and cytokinins (22, 23).

The second-highest seedling length was found in T_6 since phosphorus is necessary to stimulate early-stage root formation and promote vigorous start (cell division) to seedlings and normal growth and calcium is important for maintaining cell wall integrity, cellular metabolism and structural functions and is required for maintenance of cation-anion balance in cells. Application of 1 % straw gasification biochar increased the shoot

Table 1. Effect of combination on biopriming and biochar on physiological parameters in blackgram var. VBN 11

Treatments	Speed of germination	Germination %	Vigour index I	Dry matter production (g 10 seedlings^{-1})	Vigour index II
T_0 – Control	9.35	80	1550	0.171	13.68
T_1 – Hydropriming + Biochar at 100 g/kg of seed	10.12	80	1592	0.187	14.96
T_2 – *Bacillus* 10 % + Biochar at 100 g/kg of seed	11.80	84	1717	0.237	19.90
T_3 – *Rhizobium* 10 % + Biochar at 100 g/kg of seed	16.83	98	2278	0.310	30.38
T_4 – KRB 3 % + Biochar at 100 g/kg of seed	11.25	84	1705	0.230	19.32
T_5 – *Bacillus* 10 % + KRB 3 % + Biochar at 100 g/kg of seed	12.73	88	1789	0.240	21.12
T_6 – *Bacillus* 10 % + *Rhizobium* 10 % + Biochar at 100 g/kg of seed	15.81	96	2137	0.295	28.32
T_7 – *Rhizobium*10 % + KRB 3 % + Biochar at 100 g/kg of seed	13.58	90	1943	0.270	24.30
T_8 – *Bacillus* 10 % + *Rhizobium* 10 % + KRB 3 % + Biochar at 100 g/kg of seed	13.04	88	1869	0.260	22.88
Mean	12.72	88	1842	0.244	21.65
SEd	0.219	2.368	44.53	0.006	0.684
CD (P=0.05)	0.460**	4.975**	93.56**	0.012**	1.438**

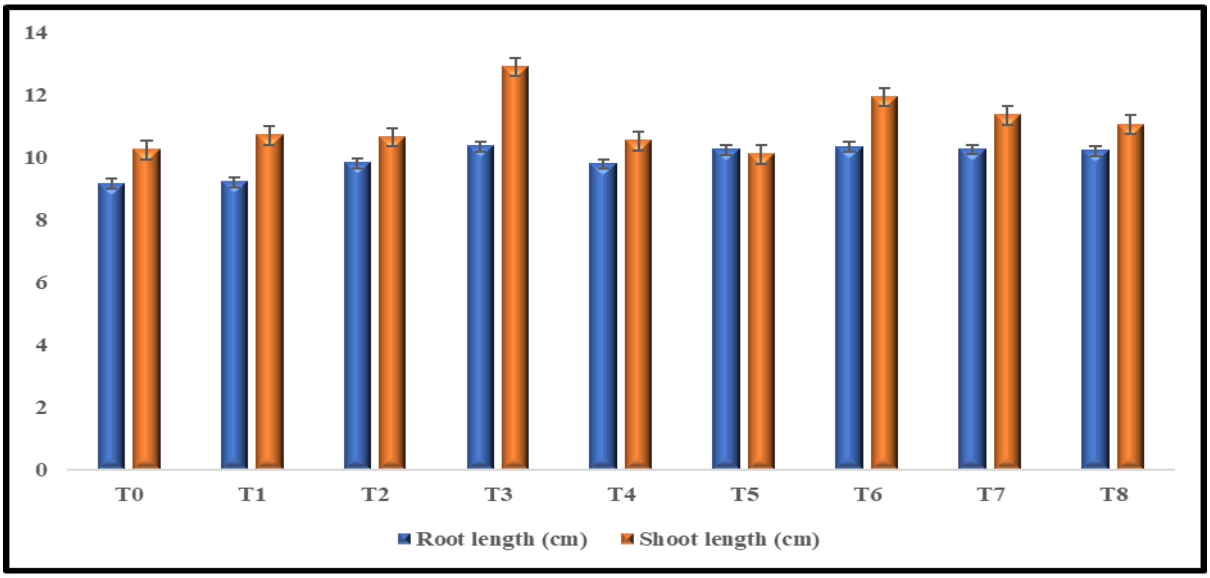

Fig. 1. Effect of combination of biopriming and biochar coating on root length and shoot length in blackgram var. VBN 11.

and root growth of barley (23). Coating grass seeds with phosphate and nitrogen promoted seedling growth. The increase in seedling growth due to liquid phosphobacteria inoculation was noticed in neem, maize, rice and wheat (24-28). The dry weight accumulation was higher in T_3 (0.310 g 10 seedlings), followed by T_6 (0.295 g/10 seedlings), compared to the control (0.171 g/10 seedlings) (Table 1).

Research indicates that the application of sludge-derived biochar stimulated the growth and weight of turf grass by nutrition enhancement in the plant (29). The increase in dry weight of rice seedling when inoculated with *Bradyrhizobia* strain UPMR48 and UPMR29 also triggers plant growth and vigour in barnyard millet (30, 31). The maximum vigour index I (2278) was recorded in T_3 and the minimum vigour index I (1550) was found in T_0 (Fig. 2). The vigour index II was higher (30.38) in T_3 and lower in the control (13.68). This may be due to the availability of nutrients and ions, more uptake of water, presence of PGR such as gibberellic acid, cytokinin and IAA (32-35). Rhizobium inoculation increased the growth, vigour and also benefited the seedling establishment and yield in rice, blackgram (35, 36). Increase in vigour while applying biochar was noticed in soybean, mango and maize (37-39).

The electrical conductivity was lower in T_3 (0.11 dS/m), followed by T_6 (0.13 dS/m) and higher in the control (T_0) (0.25 dS/m) (Fig. 3). Research indicates that an increased proportion of biochar decreases the electrical conductivity (40). Biochar-coated seeds were found to have increased dehydrogenase activity with a value of 0.593 and the lowest was observed in the control (Fig. 4). Seed coating with plant nutrient formulation in tomato seeds increased total dehydrogenase activity compared with control and seeds coated without nutrients (41). The higher peroxidase activity (0.309 mg/g/min) and catalase activity (1598 units/g) were noticed in T_3, while the lower values of 0.240 mg/g/min and 1498 units/gwere observed in control (T_0). Biopriming promotes the enzyme activity to the maximum, leading to the hydrolytic process in a faster way, as reported (42, 43).

Regarding microbial population, T_8 (*Bacillus* 10 % + *Rhizobium* 10 % + KRB 3 % + Biochar at 100 g/kg of seed) recorded higher colonisation (8.9 × 10^6 CFU/mL), followed by T_6 (8.7 × 10^6 CFU/mL). The control and hydroprimed seeds did not have any colonisation since it was not treated with any bioinoculants (Table 2). Research indicates that similar observations in rice, minor millets and greengram (17, 31, 44, 45). The increased microbial colonisation was due to the leaked exudates availability which contributed nutrients and energy for bacterial colonisation (46). Furthermore, the microbial colonisation was increased since biochar acts as a carrier for bioinoculants and increases the shelf life of bacterial count on seeds. This result was in accordance with previous results where biochar infused with *Bradyrhizobium* sp. increased the growth, biomass, nitrogen (N) and phosphorus (P) absorption and nodulation when compared to the inoculation with microbial agent alone (47).

Maximum dry matter accumulation was recorded at all stages in T_3 than in all other treatments (Table 3). It might be the reason for more availability of nutrients resulting in increased growth parameters, i.e. plant height, number of branches, leaf area and number of nodules, which ultimately turn into increased dry matter production (48, 49). Leaf area index, defined as the ratio of total leaf area to ground area used to quantify

Table 2. Effect of combination of biopriming and biochar coating on microbial population count (CFU mL^{-1} solution of seed wash) in blackgram var. VBN 11

Treatment	*Rhizobium* 10 %	*Bacillus* 10 %	KRB 3 %	Mean
T_0 – Control	0	0	0	**0**
T_1 – Hydropriming + Biochar at 100 g/kg of seed	0	0	0	0
T_2 – *Bacillus* 10 % + Biochar at 100 g/kg of seed	0	6.0 × 10^6	0	6.0 × 10^6
T_3 – *Rhizobium*10 % + Biochar at 100 g/kg of seed	8.5 × 10^6	0	0	8.5 × 10^6
T_4 – KRB 3 % + Biochar at 100 g/kg of seed	0	0	3.5 × 10^6	3.5 × 10^6
T_5 – *Bacillus* 10 % + KRB 3 % + Biochar at 100 g/kg of seed	0	6.5 × 10^6	3.7 × 10^6	5.1 × 10^6
T_6 – *Bacillus* 10 % + *Rhizobium* 10 % + Biochar at 100 g/kg of seed	10.0 × 10^6	7.3 × 10^6	0	8.7 × 10^6
T_7 – *Rhizobium* 10 % + KRB 3 % + Biochar at 100 g/kg of seed	9.6 × 10^6	0	4.0 × 10^6	6.8 × 10^6
T_8 – *Bacillus* 10 % + *Rhizobium* 10 % + KRB 3 % + Biochar at 100 g/kg of seed	14.7 × 10^6	7.7 × 10^6	4.3 × 10^6	8.9 × 10^6
Mean	10.7 × 10^6	6.9 × 10^6	3.9 × 10^6	

Table 3. Effect of combination of biopriming and biochar coating on dry weight, LAI, chlorophyll content, number of nodules and pods in Blackgram var. VBN 11

	Plant dry weight (g)		Leaf area index (LAI)		Chlorophyll content (SPAD value)		No of nodules/ plant	No of pods/ plant
Treatment	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS		
T_0 – Control	7.05	10.34	3.29	3.85	36.60	24.30	9.73	80.65
T_1 – Hydropriming	7.43	11.34	3.37	3.94	38.20	26.20	11.52	83.75
T_2 – *Bacillus* 10 % + Biochar at 100 g/kg of seed	7.96	12.24	3.66	4.18	43.70	29.10	16.81	90.28
T_3 – *Rhizobium* 10 % + Biochar at 100 g/kg of seed	9.85	15.53	4.38	4.75	55.40	35.80	36.78	120.51
T_4 – KRB 3 % + Biochar at 100 g/kg of seed	7.62	11.85	3.42	4.03	41.60	27.40	14.36	86.98
T_5 – *Bacillus* 10 % + KRB 3 % + Biochar at 100 g/kg of seed	8.05	12.52	3.85	4.25	45.30	30.30	18.21	95.24
T_6 – *Bacillus* 10 % + *Rhizobium* 10 % + Biochar at 100 g/kg of seed	9.34	15.16	4.28	4.52	51.00	35.20	32.10	112.36
T_7 – *Rhizobium*10 % + KRB 3 % + Biochar at 100 g/kg of seed	8.76	14.85	4.21	4.46	50.20	34.60	28.52	107.42
T_8 – *Bacillus* 10 % + *Rhizobium* 10 % + KRB 3 % + Biochar at 100 g/kg of seed	8.42	13.63	4.16	4.37	47.80	32.50	20.69	102.18
Mean	8.28	13.05	3.85	4.26	45.53	30.60	20.97	97.71
SEd	0.150	0.257	0.102	0.074	1.253	0.738	0.709	2.280
CD (P = 0.05)	0.319**	0.546**	0.216**	0.157**	2.656**	1.564**	1.504**	

Control *Rhizobium* **10 % + Biochar 100 g / kg**

Fig. 2. Effect of combination of biopriming and biochar coating on seedling growth in blackgram var. VBN 11.

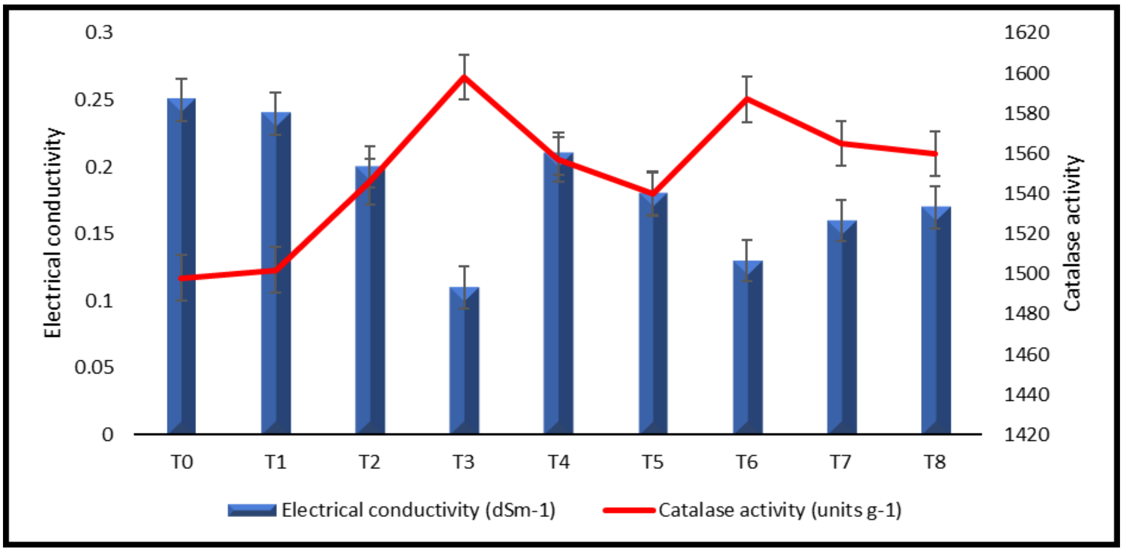

Fig. 3. Effect of combination of biopriming and biochar coating on electrical conductivity and catalase activity in blackgram var. VBN 11.

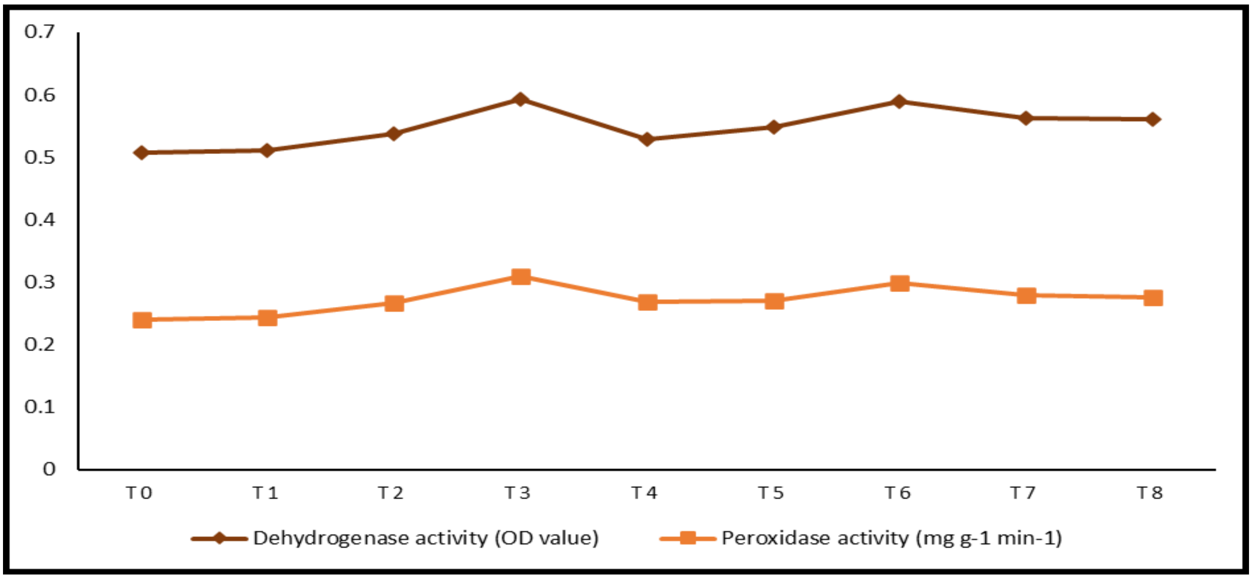

Fig. 4. Effect of combination of biopriming and biochar coating on dehydrogenase activity and peroxidase activity in blackgram var. VBN 11.

photosynthates accumulation in the sink, which in turn increases the growth of blackgram. LAI increases to the maximum after crop emergence (50). LAI was found to increase in T_3 (4.38 at 45 DAS and 4.75 at 60 DAS) compared to control (3.29 at 45 DAS and 3.85 at 60 DAS). Research indicates that the inoculation of biofertilizer had a positive impact on some parameters, such as leaf area index and biomass. Research indicates that the wood-derived biochar improved the leaf area and canopy dry weight.

The chlorophyll content in plants was determined by the cell elongation and the amount of mineral uptake in plants. In the present study, the chlorophyll content was higher in T_3 followed by T_6 over the control. The high chlorophyll content was due to the *Rhizobium*, since the nitrogen content is directly linked with photosynthetic activity, vegetative growth and dark green colour of leaves (53, 54). Research indicates that biochar made from rice husk, *Pinus densiflora* and *Quercus acutissima* wood chips promoted *Zelkova serrata* seedling growth and chlorophyll content (55).

Crop growth rate was higher in T_3 at 45 and 60 DAS (12.24 and 18.92 g/m^2/d) compared to T_0(6.72 and 9.93 g/m^2/d) (Fig. 5). The increase in crop growth rate may be due to the accelerating photosynthesis activity (56). Research indicates that bacterial inoculation resulted in increased crop growth rate (57). The number of nodules was maximum (36.78) in T_3 followed by T_6 (32.10) and the control (T_0) recorded minimum nodules (9.73) (Fig. 6). *Rhizobium* inoculation causes early nodule formation in the crown root region, this might be probably due to the effect of proliferation of nodule forming bacteria in the root system, which increases N-fixation from the atmosphere and resulting in greater plant growth and development. *Rhizobium* inoculated plants showed an increased number of nodules in green gram and soybean (58, 59). The number of pods/plant was significantly higher in T_3 (120.51) treatment over control (80.65). This increase in pods and seeds was due to the combined effect of bioinoculants and biochar. Research indicates that biochar application increased the grain yield of rice and sorghum for four growing seasons over 2 years. It leads to more nitrate reductase and nitrogenase activity in root nodules, which enhances the availability of N and P to the plant, promoting better growth and development, leading to more number of seeds per pod (61, 49).

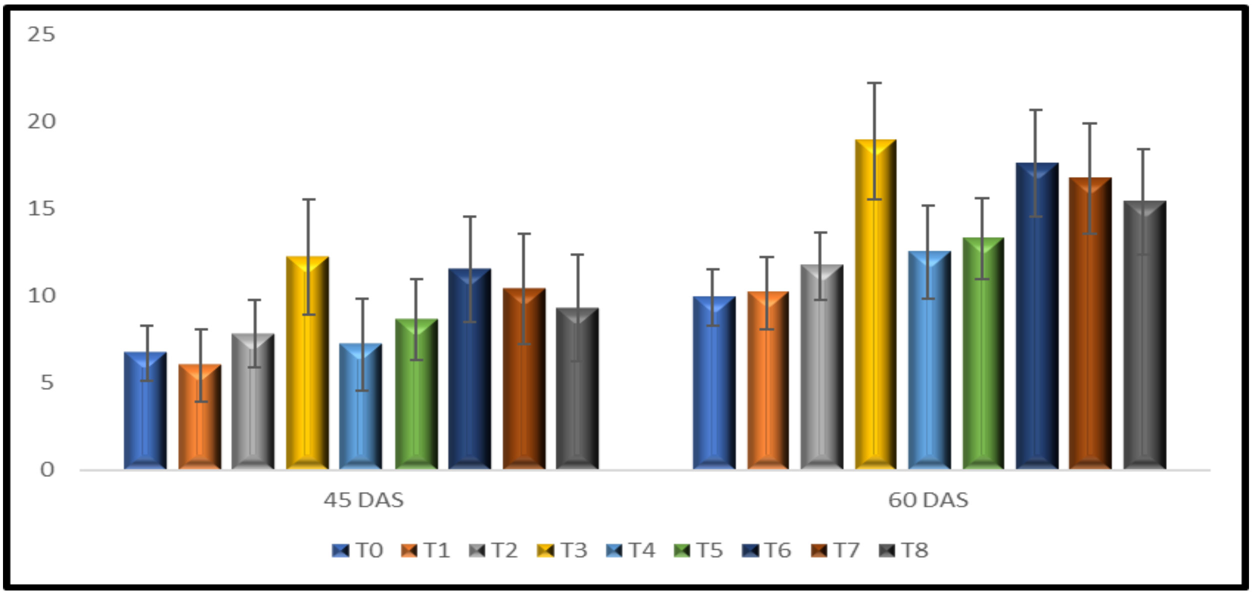

Fig. 5. Effect of combination of biopriming and biochar coating on crop growth rate (CGR) in blackgram var. VBN 11.

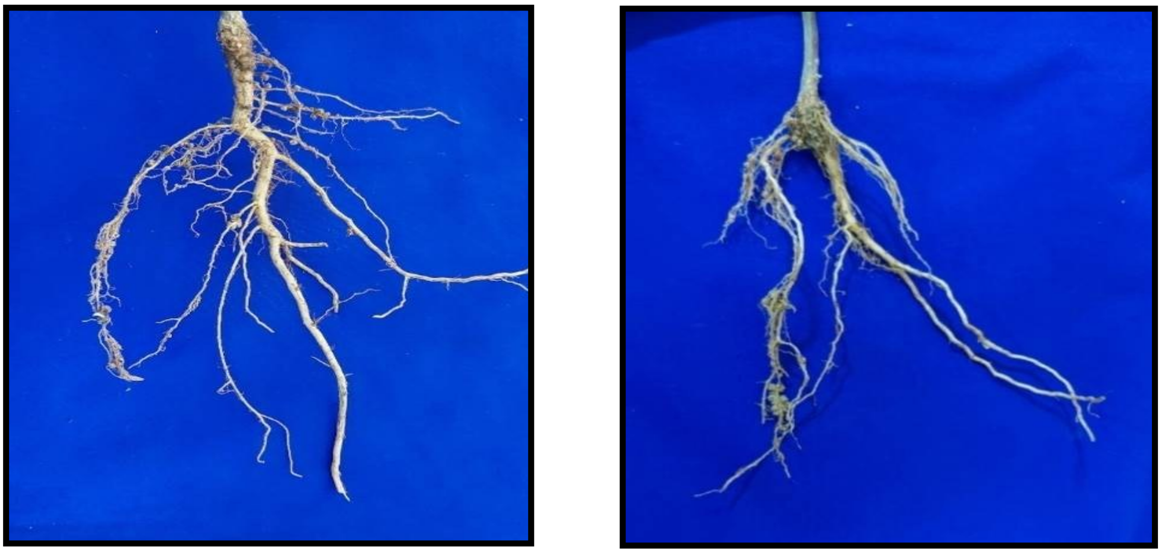

Fig. 6. Effect of combination of biopriming and biochar coating on root nodules in blackgram var. VBN 11.

The yield parameters were increased with *Rhizobium* 10 % + biochar coating 100 g/kg, followed by *Rhizobium* 10 % + *Bacillus* 10 % + biochar coating 100 g/kg, compared to the control. The seed yield per plant was higher in T_3 (33.08 g) and lower in T_0 (19.87 g) (Fig. 7). It was due to the combined effect of *Rhizobium* and biochar. The nutrient content, water holding capacity and cation exchange capacity of biochar are involved in increased yield (62). The enhanced yield was also due to nitrogen fixation of inoculated *Rhizobium* along with the native *Rhizobium* in the soil (63-65). The combination of biochar and fertiliser increased the yield in soybeans (66-68). The effect of biochar on augmented yield was registered in potato, maize and tomato (69-71).

Conclusion

The study highlights the potential of integrating biochar seed coating and biopriming as a sustainable strategy to enhance blackgram (*Vigna mungo*) production. Biochar, with its unique functional properties, served as an efficient carrier for microbial inoculums, amplifying the benefits of biopriming. Biopriming with *Rhizobium* significantly improved seed germination, vigour and resilience, resulting in superior physiological, biochemical, growth and yield parameters. The combination of *Rhizobium* (10 %) and biochar (100 g/kg) demonstrated the highest performance, markedly surpassing untreated seeds in all evaluated traits. These findings emphasise the synergistic impact of biochar and biopriming in addressing key production challenges in blackgram, including poor seed vigour and limited nutrient uptake. This integrated approach provides a scalable, eco-friendly solution for enhancing blackgram productivity and promoting sustainable cultivation practices, particularly under the growing challenges posed by climate variability and declining agricultural resources. Further research under diverse agro-climatic conditions could help validate and refine this method for wider adoption in pulse-based cropping systems.

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Authors' contributions

APB experimented and drafted the manuscript. KS, PK and AMAR participated in its design and coordination. All authors read and approved the final manuscript.

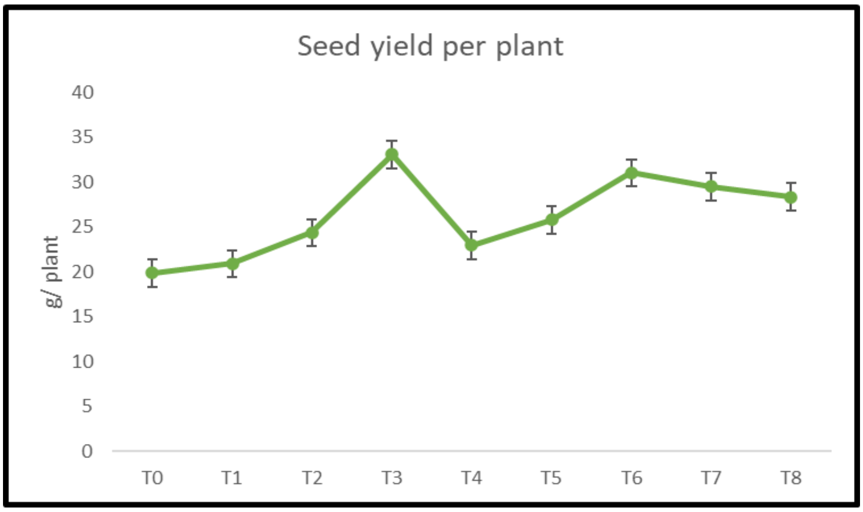

Fig. 7. Effect of combination of biopriming and biochar coating on seed yield/plant in blackgram var. VBN 11.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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