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RESEARCH ARTICLE

# Effect of Integrated Organic Seed Treatment and Foliar Nutrition on Seed Quality and Yield of Blackgram

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## ABSTRACT

Blackgram is an important pulse crop in an organic farming system capable of biological nitrogen fixation. Productivity in organic farming is lower than in conventional farming. To overcome this, seed invigoration can be achieved by combining seed priming and coating treatments, followed by foliar nutrition, to improve germination, seedling vigour, and seed yield. Blackgram seeds were primed with various organic products at different concentrations, and the best priming treatment was coated with bio-inoculants to evaluate their effect on germination, seedling vigour, field emergence, and nodulation. Chemical treatments were used as a check to compare the performance of both conventional and organic treatments. A field trial was conducted over two seasons (*Kharif* and *Rabi*) to assess the effect of organic foliar nutrition on crop growth and seed yield. Seeds primed with fermented fish extract (FFE) 5% for 3 h + coating with *Bacillus subtilis* 10 g + Rhizobium 200 g + Phosphobacteria 200 g and Tamarind Seed Polymer (TSP) 6 g /kg of seed as binder, followed by foliar spray of Panchagavya 5% at flower initiation and early pod formation stages recorded highest seed quality, crop growth and seed yield parameters.

**Keywords**: *Blackgram, Seed priming, Integrated seed treatment, Foliar spray, Organic farming*

## INTRODUCTION

Pulse crops play an important role in agriculture, dietary nutrition, and environmental sustainability. It provides low-cost, high-quality protein, nearly three times that of most cereals. Pulses are important components of organic farming systems. It is known to increase the soil's nitrogen content by fixing atmospheric nitrogen. But the crop's average productivity is far below. Blackgram is often grown in marginal, rainfed fields, with uneven germination due to low soil fertility and fluctuating moisture levels. A lack of high-quality seeds of high-yielding, disease-tolerant varieties has led to increased pest and disease outbreaks. Factors like erratic rainfall, poor soils, seed broadcasting, and the absence of proper seed invigoration treatment and nutrient management further reduce yields (Vijayan *et al.*, 2025). When crops are cultivated using organic practices compared to conventional ones, an average yield loss of 20% is predicted. The use of high-quality, vigorous seeds and a proper fertilization schedule is indispensable for

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higher productivity in both conventional and organic farming. Seeds with high germination rate and vigour establish the crop stand well, even under adverse conditions, outperforming weeds.

Seed priming is the process of hydration and dehydration in seeds, leading to the initiation of pre-germinative metabolism but preventing radicle emergence. Through seed priming, germination and seedling vigour can be increased in low-vigour seeds. Organic priming has the potential to increase seed quality parameters and yield compared with hydro priming (Vanitha and Kathiravan, 2022). Seed coating with bio-inoculants such as Rhizobium, Phosphorus Solubilizing Bacteria (PSB), and *Bacillus* spp. can improve germination, seedling vigour, soil fertility, and yield by efficiently mobilizing nutrients and alleviating stress conditions, yielding better results in combination than in individual treatments (Sheteiwy *et al.*, 2021; Kavitha and Srimathi, 2022). Synthetic polymers are used as binders for coating, but are less degradable and can harm bioinoculants, reducing their population. Tamarind Seed Polysaccharide (TSP), rich in gelatin, serves as a natural binder and organic alternative to synthetic polymers for coating treatments. After crop establishment, it is vital to provide the crop with the proper nutrients. Foliar application of nutrients at critical growth stages enhances dry matter accumulation, root and shoot development, and leaf area, thereby contributing to higher yields. The use of organic products like panchagavya, Fermented Fish Extract (FFE), Egg fermented extract (EFE), Seaweed Extract (SWE), Vermiwash (VW), and Beejamrutha, obtained from the fermentation of on-farm waste materials, is suitable for organic farming systems and offers a sustainable, low-cost alternative to synthetic fertilizers. Therefore, this study aimed to assess the effect of integrated organic seed treatment and foliar nutrition with various organic products on seed quality, seedling vigour, and seed yield in blackgram.

## MATERIALS AND METHODS

The laboratory experiments were carried out at the Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore. Medium vigour seeds of blackgram cv. VBN 11 was collected from the Department of Pulses, TNAU, Coimbatore for the study. Organic products such as FFE, Panchagavya, Beejamrutha, and VW were obtained from the Nammazhvar Organic Farming Research Centre, TNAU, Coimbatore.

### *Preparation of organic products*

FFE is produced by fermenting chopped fish waste with sugar and beneficial microbes in airtight containers for 15 days. Panchagavya is produced by fermenting a mixture of five cow-derived products like dung, urine, milk, curd, and ghee along with water and jaggery over 7 to 21 days under aerobic conditions. Beejamrutha is prepared by mixing cow dung, cow urine, lime, and a small amount of soil with water and fermenting the mixture for 24 hours. VW is a liquid extract obtained by passing water through a worm culture during the vermicomposting process. EFE was prepared by soaking eggs in lemon juice for 10 days, then smashing and mixing with jaggery syrup and leaving it for another 10 days before use as a spray. SWE is prepared by shade-drying the seaweed species Turbinaria conoides for 4 days and oven-drying at 60 °C for 12 hours. The dried product was ground to a coarse powder, mixed with distilled water (1:20 w/v), and autoclaved at 121 °C for 30 minutes. The extract was filtered, cooled, and taken at 100% concentration, then diluted to the required quantity.

### *Effect of seed priming with organic products on seed quality parameters*

The blackgram seeds were soaked for 3 h in different organic products such as SWE (1%, 1.5%, 2%), FFE (5%, 10%, 15%), Panchagavya (5%, 10%, 15%), Beejamrutha (100%) and VW (5%, 10%, 15%) along with Control (no priming), Hydropriming and Zinc sulphate ($ZnSO_4$) at 100 ppm as chemical treatment to assess the comparative performance. Then the seeds were shade-dried to their original moisture content and evaluated for physiological and biochemical seed quality characters. Physiological parameters observed in the study were germination percentage (ISTA, 2019), root length (cm), shoot length (cm), dry matter production (mg 10 seedling$^{-1}$) (ISTA, 2019), Speed of germination (Maguire, 1962), and seedling vigour index (Abdul Baki and Anderson, 1973). Biochemical parameters such as protein content (Ali-Khan and Youngs, 1973), dehydrogenase activity (OD value) (Kittock and Law, 1968), and α-amylase activity (mg maltose/min) (Paul *et al.*, 1970).

### *Effect of integrated seed treatment on seed quality parameters*

Based on the findings, the best-performing priming treatment was selected and carried forward to the subsequent experiment, where it was combined with biofertilizer coating and chemical treatments to assess the comparative effect of integrated seed treatments. Integrated Organic Seed (IOS) treatment includes Priming with FFE 5% + coating with *Bacillus subtilis* 10 g + *Rhizobium* 200 g + Phosphobacteria 200 g/kg of seed using TSP 6 g/kg of seed as binder. TSP was prepared from tamarind kernel powder following the method for pharmaceutical hard capsule preparation. Integrated Chemical Seed (ICS) treatment includes priming with $ZnSO_4$ 100 ppm + coating (Polymer 3 ml + Imidacloprid 17.8% SL 5 ml + Carbendazim 25% WP 2 g + *Rhizobium* 200 g + Phosphobacteria 200 g /kg of seed) using synthetic white polymer as binder for subsequent layers (Figure 1). Seven different combinations of treatments, including a control, were formulated in four replications using a Completely Randomized Design to assess the effect of integrated seed treatment on physiological seed quality parameters (Table 1). The pot culture studies were conducted to assess the effect of integrated seed treatment under field conditions by evaluating field emergence (%), leghaemoglobin concentration (Wilson and Reisenauer, 1963) in root nodules, and the number of nodules/plant.

### *Metabolite profiling of root exudates*

Seedlings from the pot culture experiment were uprooted on the seventh day, and roots were washed with deionized water to remove soil. They were then transferred to glass tubes containing 50 mL of Hoagland’s nutrient solution (Hoagland and Arnon, 1950), with sponges placed at the tube mouth to support seedlings and ensure that only roots contacted the solution. Root exudates were collected 15 days post-transfer, extracted with an equal volume of ethyl acetate (1:1, v/v), and shaken at 150 rpm for 12 h. The ethyl acetate layer was separated, evaporated using a rotary evaporator, and the residue was dissolved in 1 mL of MS-grade methanol for metabolite identification through GC-MS.

### *Field experiment*

A field experiment was carried out for two seasons in a wetland field at TNAU, Coimbatore (*Kharif*) and on an individual farm in Pappampatti, Coimbatore (*Rabi*) during the year 2023-2025. The seeds were treated with the best-performing treatment from pot culture studies before sowing. The field trial was set up as a one-factorial experiment following a Randomized

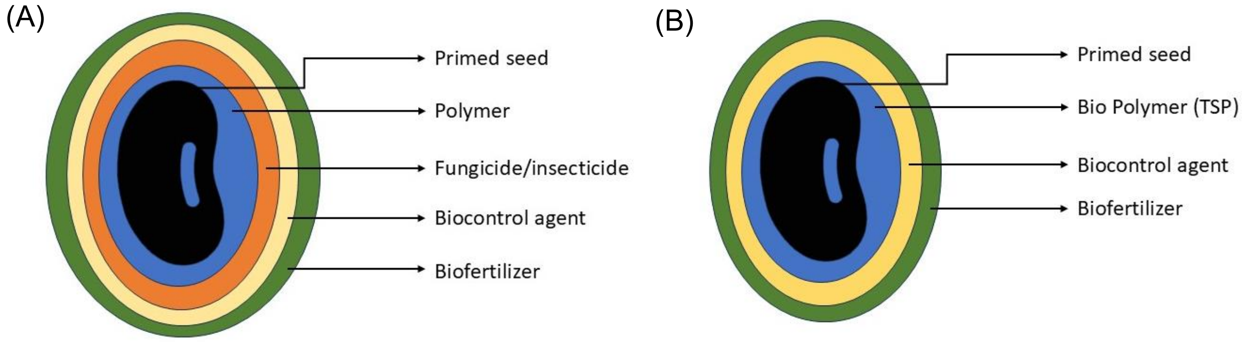


**Figure 1: illustration of Integrated chemical seed treatment (A) and Integrated organic seed treatment (B)**

**Table 1. Integrated seed treatment experiment details**

| | |
|---|---|
| $T_1$ | - Control (no treatment) |
| $T_2$ | - Priming with $ZnSO_4$ 100 ppm + coating (Polymer 3 ml + Imidacloprid 5 ml + Carbendazim 2 g + Rhizobium 200 g + Phosphobacteria 200 g /kg of seed) (ICS treatment) |
| $T_3$ | - Priming with FFE 5% (no coating) |
| $T_4$ | - Coating with *Bacillus subtilis* 10 g + *Rhizobium* 200 g + Phosphobacteria 200 g /kg of seed |
| $T_5$ | - $T_3$ + $T_4$ |
| $T_6$ | - $T_4$ + Tamarind Seed Polymer (TSP) 6 g /kg of seed |
| $T_7$ | - $T_5$ + Tamarind Seed Polymer (TSP) 6 g /kg of seed (IOS treatment) |

Block Design with three replications (plot size of 12 m²). The plots were sprayed with two doses of various organic products: Panchagavya (3%, 5%), SWE (5%, 10%), FFE (2%, 4%), and EFE (2%, 4%) at the flower initiation and early pod-formation stages. Fertilization was carried out entirely under organic conditions, with the application of 1.25 tonnes/ha of vermicompost and manual weed control. After two foliar sprays, growth parameters, including Leaf Area Index (LAI), Specific leaf weight (SLW), and plant height at 45 and 60 Days After Sowing (DAS), were recorded. Soluble Leaf Protein (SLP) (Lowry *et al.*, 1951) and chlorophyll content (SPAD value) were estimated during the pod maturation stage. Plant biomass was assessed by uprooting and shade-drying whole plant samples for 2 days, followed by oven-drying at 80 ˚C for 16 h. Yield attributes, such as pods/plant, number of filled seeds/pod, Seed yield/hectare, and hundred-seed weight were recorded at harvest. Seed yield obtained from the plots was converted into a yield/ha. Data from both seasons were analyzed on a pooled basis.

### *Data analysis*

The data were analysed using AGRES (Agricultural Research Statistical Software), and the variation was examined using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. Before analysis, percentage numbers were converted to arcsine values. The significant difference was examined at the $P < 0.05$ level. Additionally, Microsoft Excel was used to create the graphical depiction (Office 2019, Microsoft Corporation, USA).

## RESULTS AND DISCUSSION

### *Effect of seed priming with organic products*

The data revealed that the highest germination (96%) was recorded in seeds primed with FFE at 5% and ZnSO4 at 100 ppm, compared with the control (83%) (Table 2). The dry matter production and SOG in FFE 5% primed seeds were higher (292 mg 10 seedling-1 and 21.67), followed by ZnSO4 100ppm (288 mg 10 seedling$^{-1}$ and 21.16), compared to the control (265 10 seedling$^{-1}$ and 15.72). The root length and shoot length were higher (19.23 and 18.58 cm) in seed priming with $ZnSO_4$ 100 ppm, followed by FFE 5% (18.78 and 18.44 cm), compared to the control (13.77 and 13.15 cm) (Table 2). Further $ZnSO_4$ 100 ppm priming also recorded a higher vigour index (3630) and was on par with FFE 5% (3574) compared to the control (2226). Seeds primed with $ZnSO_4$ 100

**Table 2. Effect of seed priming with bioformulations on seed quality characteristics in blackgram seeds cv.VBN 11**

| Treatments | Germination (%) | Speed of germination | Root length (cm) | Shoot length (cm) | Dry matter production (mg 10 seedling$^{-1}$) | Vigour index |
|---|---|---|---|---|---|---|
| Control | 83(65.65)$^{e}$ | 15.72$^{h}$ | 13.77$^{j}$ | 13.15$^{i}$ | 265$^{h}$ | 2226$^{g}$ |
| Hydropriming | 88(69.73)$^{cd}$ | 18.83$^{de}$ | 15.67$^{de}$ | 14.17$^{gh}$ | 269$^{gh}$ | 2626$^{e}$ |
| $ZnSO_4$ 100 ppm | 96(78.47)$^{a}$ | 21.16$^{a}$ | 19.23$^{a}$ | 18.58$^{a}$ | 288$^{ab}$ | 3630$^{a}$ |
| SWE 1% | 85(67.21)$^{de}$ | 17.5$^{g}$ | 14.40$^{i}$ | 14.12$^{h}$ | 267$^{h}$ | 2434$^{f}$ |
| SWE 1.5% | 89(70.63)$^{bc}$ | 17.83$^{fg}$ | 15.41$^{ef}$ | 14.73$^{fgh}$ | 279$^{def}$ | 2693$^{de}$ |
| SWE 2% | 89(70.63)$^{bc}$ | 18.17$^{efg}$ | 15.40$^{efg}$ | 14.81$^{efg}$ | 280$^{de}$ | 2699$^{cde}$ |
| FFE 5% | 96(78.47)$^{a}$ | 21.67$^{a}$ | 18.78$^{b}$ | 18.44$^{a}$ | 292$^{a}$ | 3574$^{a}$ |
| FFE 10% | 88(69.73)$^{cd}$ | 18.83$^{de}$ | 15.24$^{fgh}$ | 15.02$^{def}$ | 274$^{fg}$ | 2663$^{de}$ |
| FFE 15% | 87(68.87)$^{cd}$ | 18.00$^{efg}$ | 15.06$^{h}$ | 14.98$^{ef}$ | 268$^{h}$ | 2605$^{de}$ |
| Panchagavya 5% | 87(68.87)$^{cd}$ | 17.50$^{g}$ | 15.21$^{fgh}$ | 15.44$^{cde}$ | 268$^{gh}$ | 2657$^{de}$ |
| Panchagavya 10% | 92(73.57)$^{b}$ | 18.67$^{ef}$ | 17.49$^{c}$ | 17.71$^{b}$ | 286$^{bc}$ | 3239$^{b}$ |
| Panchagavya 15% | 89(70.63)$^{bc}$ | 20.17$^{b}$ | 14.56$^{i}$ | 15.26$^{cde}$ | 275$^{ef}$ | 2665$^{de}$ |
| Beejamrutha 100% | 89(70.63)$^{bc}$ | 19.83$^{bc}$ | 15.85$^{d}$ | 15.77$^{c}$ | 285$^{bc}$ | 2826$^{c}$ |
| VW 5% | 89(70.63)$^{bc}$ | 18.83$^{de}$ | 15.07$^{h}$ | 15.46$^{cde}$ | 282$^{cd}$ | 2728$^{cde}$ |
| VW 10% | 89(70.63)$^{bc}$ | 18.67$^{bcd}$ | 15.38$^{efg}$ | 15.69$^{cd}$ | 285$^{bc}$ | 2776$^{c}$ |
| VW 15% | 92(73.57)$^{b}$ | 18.94$^{cde}$ | 17.18$^{c}$ | 17.62$^{b}$ | 286$^{bc}$ | 3202$^{b}$ |

(Figures in parentheses indicate arcsine values) ppm showed higher protein content (22.63%) and α-amylase activity (13.03 mg maltose/min), followed by FFE 5% (22.71% and 13.14 mg maltose/min), compared to the control (21.85% and 12.38 mg maltose/min) (Figure 2). The highest dehydrogenase (2.95 OD) was recorded in FFE 5% compared to the control (1.43 OD) (Figure 2).

***Effect of integrated seed treatment***

The highest germination (92%) was recorded in the ICS and IOS treatments compared with the control (80%). SOG was higher (21.17) in seed primed with FFE 5% followed by the IOS treatment (19.0) than in the control (15.83). The root length, shoot length, and vigour index were higher (15.29 cm, 19.12 cm,

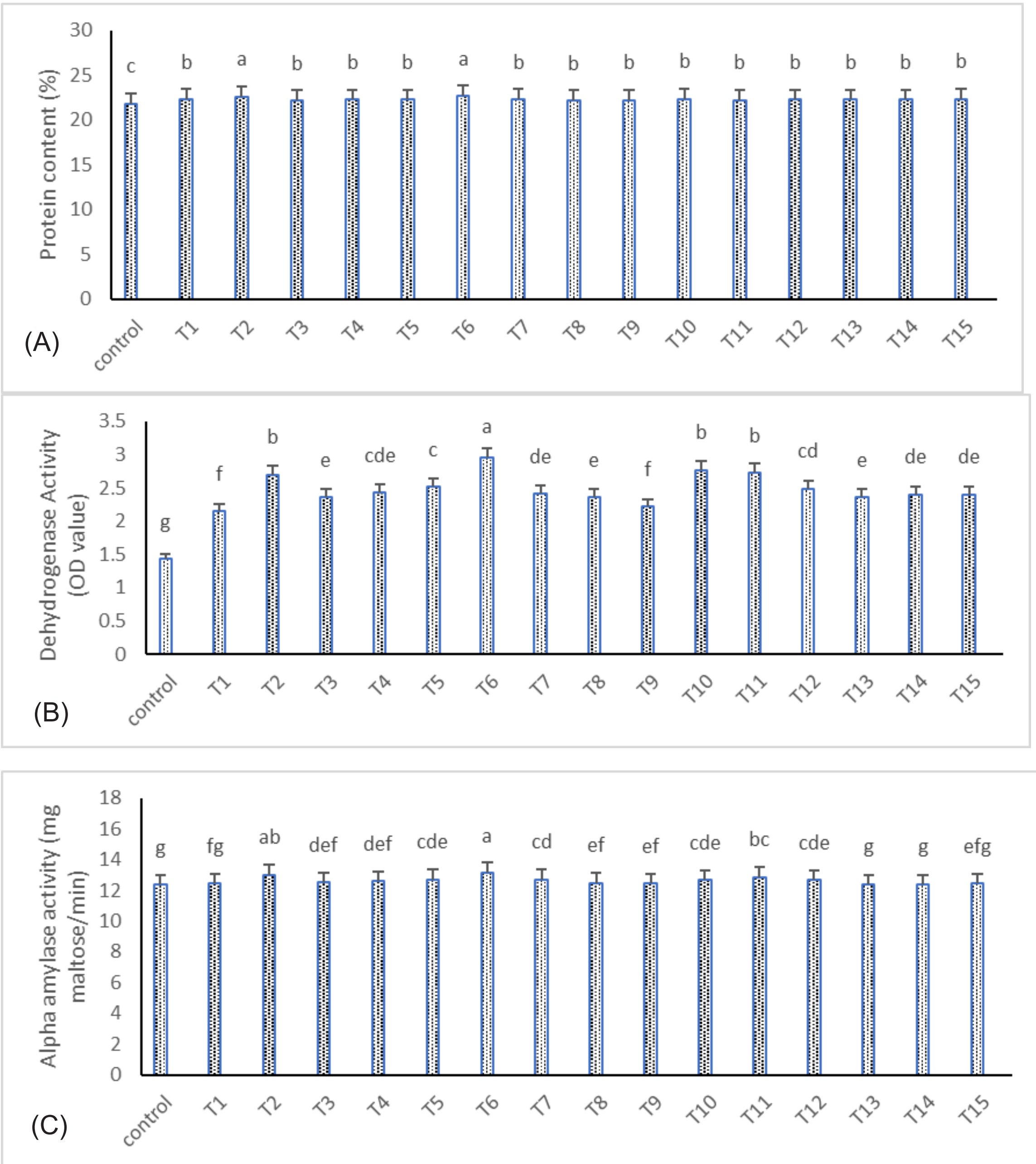


**Figure 2. Effect of seed priming with organics on protein content (%) (A), dehydrogenase (B), and α-amylase activity (C) in blackgram cv. VBN 11. T0 – Control, T1 – Hydropriming, T2-ZnSO4 100 ppm, T3- SWE 1%, T4 - SWE 1.5%, T5 - SWE 2%, T6 – FFE 5%, T7 - FFE 10%, T8 - FFE 15%, T9 - Panchagavya 5%, T10 - Panchagavya 10%, T11 - Panchagavya 15%, T12 – Beejamrutha 100%, T13 – VW 5%, T14 - VW 10%, T15 - VW 15%.**

and 3165) in ICS treatment followed by IOS treatment (14.85 cm, 19.00 cm, and 3114) over control (13.02 cm, 17.23 cm, and 2420) (Table 3). Dry matter production was higher (316 mg 10 seedling$^{-1}$) in the IOS treatment, followed by the ICS treatment (301 mg 10 seedling-1), than the control (254 mg 10 seedling$^{-1}$). Among organic treatments, the IOS treatment showed enhanced physiological seed quality parameters. In pot culture studies, field emergence was higher (89%) in IOS treatment, followed by ICS treatment, over control (79%) (Table 4). The highest number of nodules/plant (50.67) was recorded in the IOS treatment, followed by ICS (44.67), and control (25.0). The leghaemoglobin content was higher (3.05 mg/g fresh weight of nodule) in the IOS treatment than in the control (2.03 mg/g fresh weight of nodule) (Table 4).

***Metabolite profiling of root exudates***

GC-MS analysis of root exudates collected from 15-day-old seedlings revealed a significant difference in compounds released by seedlings treated with IOS compared to control seeds. A total of 57 compounds were identified in root exudates, belonging to various categories such as sugars, fatty acids, carboxylic acids, phenolic compounds, ethers, amino acids, aldehydes, organosilicons, and other compounds (Figure 4). The untreated root exudates contain 36

**Table 3. Effect of integrated seed treatment on seed quality characteristics in blackgram seeds cv.VBN 11**

| Treatments | Germination (%) | Speed of germination | Root length (cm) | Shoot length (cm) | Dry matter production (mg 10 seedling$^{-1}$) | Vigour index |
|---|---|---|---|---|---|---|
| Control ($T_1$) | 80 (63.44)$^{d}$ | 15.83$^{d}$ | 13.02$^{d}$ | 17.23$^{d}$ | 254$^{e}$ | 2420$^{f}$ |
| ICS treatment ($T_2$) | 92 (73.57)$^{a}$ | 18.17$^{c}$ | 15.29$^{a}$ | 19.12$^{a}$ | 301$^{b}$ | 3165$^{a}$ |
| Priming with FFE 5% (no coating) ($T_3$) | 88 (69.73)$^{b}$ | 21.17$^{a}$ | 14.48$^{bc}$ | 18.36$^{b}$ | 279$^{c}$ | 2890$^{c}$ |
| Coating with *Bacillus subtilis* 10 g + *Rhizobium* 200 g + Phosphobacteria 200 g /kg of seed ($T_4$) | 81 (64.16)$^{d}$ | 18.50$^{bc}$ | 13.42$^{d}$ | 17.37$^{d}$ | 263$^{d}$ | 2493$^{e}$ |
| $T_3$ + $T_4$ ($T_5$) | 88 (69.73)$^{b}$ | 18.83$^{b}$ | 14.51$^{bc}$ | 18.41$^{b}$ | 280$^{c}$ | 2897$^{c}$ |
| $T_4$ + TSP 6 g /kg of seed ($T_6$) | 85 (67.21)$^{c}$ | 18.67$^{bc}$ | 14.11$^{c}$ | 17.96$^{c}$ | 278$^{c}$ | 2725$^{d}$ |
| $T_5$ + TSP 6 g /kg of seed (IOS treatment) ($T_7$) | 92 (73.57)$^{a}$ | 19.00$^{b}$ | 14.85$^{ab}$ | 19.00$^{a}$ | 316$^{a}$ | 3114$^{b}$ |

(Figures in parentheses indicate arcsine values)

**Table 4. Effect of integrated seed treatment on the field performance of blackgram seeds, cv.VBN 11**

| Treatments | Field emergence (%) | Leghemoglobin (mg/g fresh weight of nodule) | Number of nodules/ plants |
|---|---|---|---|
| Control ($T_1$) | 79(62.72)$^{d}$ | 2.03$^{d}$ | 25.00$^{e}$ |
| ICS treatment ($T_2$) | 88(69.73)$^{ab}$ | 2.67$^{b}$ | 44.67$^{b}$ |
| Priming with FFE 5% (no coating) ($T_3$) | 85(67.22)$^{c}$ | 2.25$^{cd}$ | 34.67$^{d}$ |
| Coating with *Bacillus subtilis* 10 g + *Rhizobium* 200 g + Phosphobacteria 200 g /kg of seed ($T_4$) | 81(64.16)$^{d}$ | 2.35$^{c}$ | 36.67$^{d}$ |
| $T_3$ + $T_4$ ($T_5$) | 86(68.03)$^{bc}$ | 2.73$^{b}$ | 43.33$^{bc}$ |
| $T_4$ + TSP 6 g /kg of seed ($T_6$) | 81(64.16)$^{d}$ | 2.16$^{cd}$ | 41.33$^{c}$ |
| $T_5$ + TSP 6 g /kg of seed (IOS treatment) ($T_7$) | 89(70.63)$^{a}$ | 3.05$^{a}$ | 50.67$^{a}$ |

(Figures in parentheses indicate arcsine values)

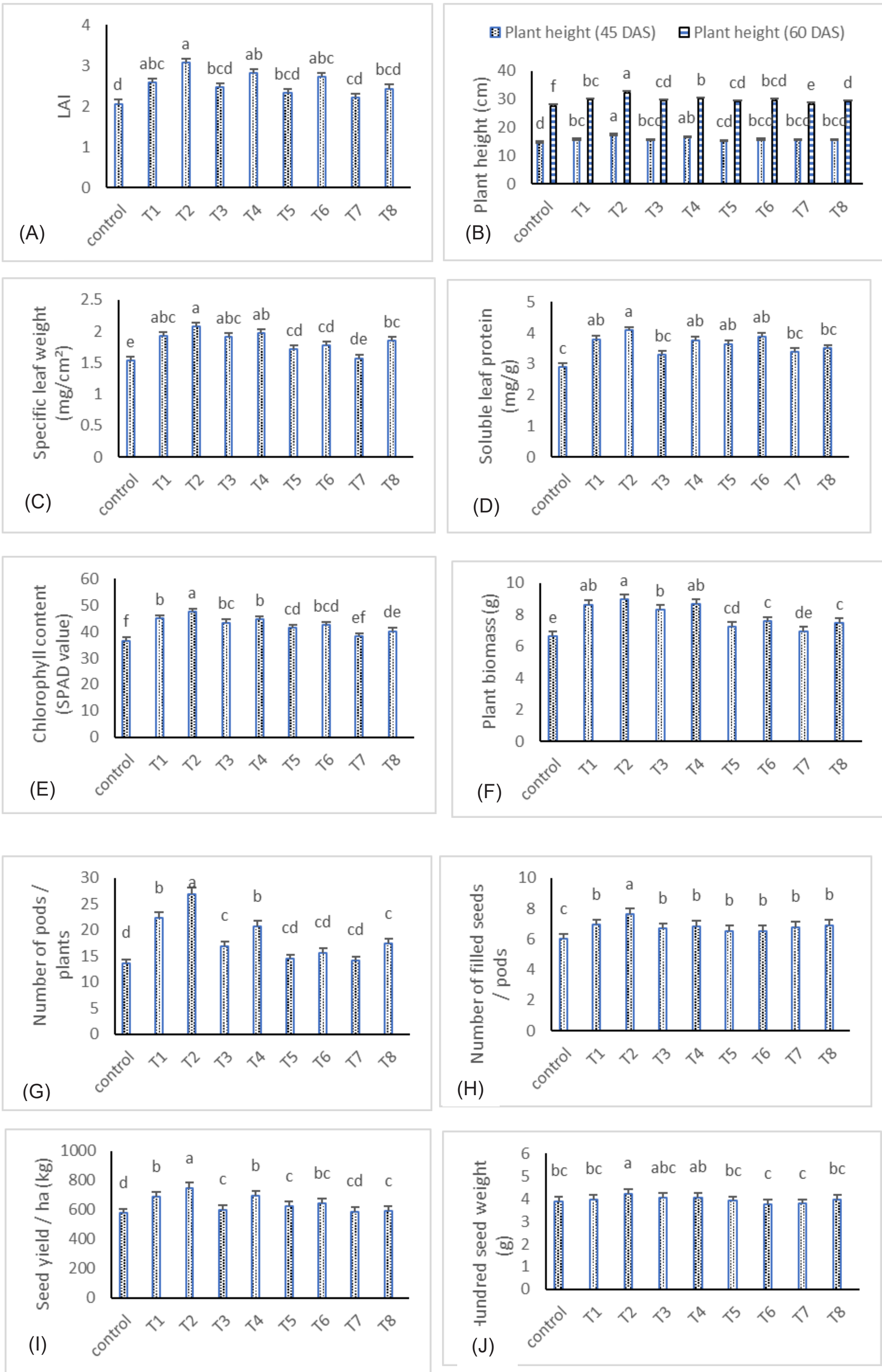


**Figure 3. Effect of foliar spray treatments on crop growth and yield parameters**;

LAI (A), plant height at 45DAS and 60DAS (B), SLW (C), SLP (D), chlorophyll content (E), Plant biomass (F), number of pods/plants (G), number of filled seeds/pod (H), Seed yield/hectare (I) and hundred seed weight (J). T1 - Panchagavya 3%, T2 – Panchagavya 5%, T3 - SWE 5%, T4 - SWE 10%, T5 - FFE 2% T6 - FFE 4%, T7 - EFE 2% and T8 - EFE 4%.

compounds, and those from the IOS treatment contain 32. Among these, 11 compounds were found in both the root exudates. Untreated root exudates have a higher proportion of sugars and ethers than those under IOS treatment. But all other categories, such as fatty acids, carboxylic acids, phenolic compounds, amino acids, aldehydes, and organosilicons, were higher in IOS treatment. Significant variations in the metabolite expression patterns between the two treatments were shown by the heatmap analysis (Figure 5).

### *Effect of foliar spray treatments on crop growth and seed yield*

Figure 3 shows that the foliar spray of Panchagavya 5% recorded highest in growth parameters like LAI (3.07), SLW (2.07 mg/cm$^2$), plant height at 45DAS (17.46 cm) and 60DAS (32.29 cm), SLP (4.08 mg/g) and chlorophyll content (47.62) and Plant biomass (8.97 g), based on pooled data from two seasons. Yield attributes such as pods/plant (26.79), number of filled seeds/pod (7.6), Seed yield/hectare (795 kg), and hundred seed weight (4.23 g) were also recorded as highest in plots treated with a foliar spray of Panchagavya 5%. The growth and yield parameters vary between seasons soybean yields vary between years. Therefore, the results represent two-season averages derived from a pooled analysis.

Primed seeds exhibit faster germination, driven by early enzyme activation and reserve mobilization, thereby enhancing seedling vigour compared to controls. Enzymes such as α-amylase hydrolyse stored carbohydrates, enabling the efficient utilization of food reserves during germination. In our study, FFE at 5% and ZnSO4 at 100 ppm showed higher germination. Kavitha and Srimathi (2022) reported that $ZnSO_4$ priming improved root efficiency and metabolic activity by greater hydration of colloids, increased inbound water content, higher viscosity and elasticity of protoplasm, resulting in a more efficient root system, and enhanced IAA synthesis. It also increased protein content and α-amylase activity, likely due to elevated nitrate reductase activity(Choudhary *et al.*, 2021). FFE provides essential nutrients and amino acids that promote protein synthesis, enhancing germination. It also supplies glutamate, a proline precursor, aiding germination and crop growth under stress conditions (Horii *et al.*, 2007a). Therefore, the increase in physiological seed quality parameters may be due to precursors in FFE, which enhance lipid synthesis through the acetyl-CoA pathway for fatty acid production, providing energy for cell division and cell elongation (Siripongvutikorn *et al.*, 2016). Dehydrogenase activity serving as an index of respiration and metabolism in cells, increasing with enhanced α-amylase activity, both of which indicate the utilization of stored reserves and the level of vigour and viability in seeds. Among organics, seeds primed with FFE 5% performed on par with the chemical seed-priming treatment $ZnSO_4$ 100 ppm. Seed with richer phenolic profiles, such as legumes, respond to glutamic acid supplementation by FEE, leading to possible stimulation of PPP and providing the necessary energy for germination and seedling growth (Horii *et al.*, 2007b). This might be a reason for improved performance of FFE primed seeds when compared to other organics.

Biofertilizer seed treatments improved germination and growth by releasing plant hormones and enhancing nutrient mobilization. Adding fungicide to bio-inoculant may have a negative influence on the population of bio-inoculants, but Raja *et al.* (2019) reported that fungicide-treated seeds recorded a minimum population of 1 × 10^4 cfu $g^{-1}$, which might be enough to build up in the rhizosphere and positively influence the plant growth. Higher germination, root and shoot length, and vigour index may be due to co-inoculation with *Rhizobium* and *Bacillus* spp., which promoted seedling growth under stress by enhancing IAA and other growth hormone production (Miljakovic *et al.*, 2022). TSP is an effective binder due to its adhesive, fast-dissolving nature, rich carbohydrate and amino acid content, and antioxidant properties that help scavenge reactive oxygen species (Geethalaxmi *et al.*, 2024).

The increased nodule number may be due to co-inoculation with *Rhizobium* and *Bacillus* spp., which synergistically enhanced IAA production, promoting cell elongation and cortical proliferation. (Sibponkrung *et al.*, 2020). Co-inoculation also improved nodule nitrogenase activity and increased soil nitrogen content by regulating the nitrogen cycle (Xing *et al.*, 2022). Nitrogenase, the principal enzyme of biological nitrogen fixation, is extremely oxygen-sensitive, while symbiotic bacteria need oxygen to generate energy. Legumes manage this by an oxygen diffusion barrier in the nodule cortex and by high levels of leghaemoglobin in infected cells, which control oxygen concentrations by binding surplus $O_2$ (Zhou *et al.*, 2023). Thus, the higher leghaemoglobin content was due to co-inoculation of Rhizobium and PSB, which promoted effective nodulation and increased nutrient availability. Similar findings were reported by Kumawat *et al.* (2022) in soybean, and in chickpea, co-inoculation of rhizobium and PSB produces higher leghaemoglobin content in nodular tissues compared to individual inoculation (Tagore *et al.*, 2013). Among organic treatments, IOS showed improved physiological seed quality, likely due to the combined effects of 5% FFE priming and co-inoculation with Bacillus subtilis, Rhizobium, and Phosphobacteria, using TSP as a binder.

Root exudates are crucial for plant-microbe interactions. Among 11 universal compounds, glyceraldehyde-meto-2TMS was most prevalent. As a key glycolysis intermediate, its deficiency disrupts root growth and metabolic balance in *Arabidopsis thaliana*. (Munoz-Bertomeu *et al.*, 2009). Glyoxylic acid, another prevalent compound, is vital to the glyoxylate cycle and photorespiration. Arachidonic acid is an allelopathic signal for rhizosphere bacteria but inhibits nitrifying bacteria (Wang *et al.*, 2023). Lipoic acid, with antioxidant and metal-chelating properties, was also high (Vega *et al.*, 2022). IOS-treated root exudates had twice the levels of glyceraldehyde-meto-2TMS, glyoxylic acid, and lipoic acid compared to untreated seeds, although untreated exudates contained more arachidonic acid. Root exudates of IOS treatment contained nonanoic acid, palmitoleic acid, and juniperic acid in a novel combination. Nonanoic acid (pelargonic acid) induces MAPKs involved in plant defense (Dombrowski and Martin, 2014). Palmitoleic acid, a palmitic acid derivative, can inhibit soil-borne diseases and promote plant growth by modulating the rhizosphere microbial community (Ma *et al.*, 2021). Juniperic acid, a cutin monomer, can be involved in the attraction of beneficial microbes (Cao *et al.*, 2023). These findings suggest that the root exudates from IOS treatment may possess the ability to act as an antimicrobial antioxidant and alleviate stress conditions. It also acts as a carbon source and as a signalling compound that induces beneficial soil microbe colonization.

Two foliar sprays were given at the flower initiation and early pod formation stages to ensure effective utilization of the spray formulations. The pooled data suggest that growth parameters, such as LAI and SLW, were higher in the Panchagavya 5% treatment. Loganathan (2014) suggested that Panchagavya contains growth hormones such as Auxins and gibberellins, which favour rapid cell division and elongation, leading to higher LAI and SLW. Auxin in Panchagavya promotes cell division and elongation in axillary buds, enhancing plant height and leaf area (Sutar *et al.*, 2019). It results in increased chlorophyll synthesis and nitrogen assimilation, leading to higher SLP and chlorophyll content in the leaf. Panchagavya spray likely boosted plant biomass by enhancing nutrient absorption and mobilization, thereby improving yield traits. Panchagavya promotes prolonged stomatal opening, increasing $CO_2$ diffusion and photosynthesis, and increasing the efficiency of source-to-sink nutrient mobilisation. These results are in accordance with findings of Sakthivel and Dhanapal (Sakthivel *et al.*, 2022) and Priyanka *et al.* (2024). Vinutha *et al.* (2023) reported that economically beneficial returns were higher with panchagavya than with FFE and EFE treatments.

## CONCLUSION

Blackgram seeds primed with FFE 5% recorded enhanced germination and seedling vigour among the organics. Priming with FFE 5% + coating with *Bacillus subtilis* 10 g + *Rhizobium* 200 g + Phosphobacteria 200 g/kg of seed, with TSP 6 g/kg of seed as binder, outperformed other organic treatments in both laboratory and pot culture studies. Foliar spray of Panchagavya 5% at flower initiation and early pod formation stages enhanced crop growth and recorded maximum seed yield. Thus, integration of seed priming with FFE 5% and coating with bioinoculants (*Bacillus subtilis* 10 g, *Rhizobium* 200 g and Phosphobacteria 200 g /kg of seed) using tamarind seed polymer 6 g /kg of seed as binder combined with foliar spray of Panchagavya 5% could be utilized as a sustainable practice to improve the germination, seedling vigour and yield traits of blackgram.

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### *Ethics Statement:*

This study does not involve human or animal subjects; therefore, ethical approval is not applicable.

### *Competing Interests:*

The authors declare that they have no competing interests.

***Data Availability:***

All data about the research have been presented in tables and figures.

***Author Contributions:***

All authors contributed to the research work, reviewed, and edited the manuscript.

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