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RESEARCH ARTICLE

Enhancing the yield of black gram (*Vigna mungo* L.) using organic seaweed extract

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Abstract

Seaweed extracts are gaining attention due to growing popularity of organic food and sustainable agricultural practices. Owing to their sustainability and eco-friendly nature, seaweed extracts are widely utilized in organic agriculture. This study aims to enhance black gram yield and maintain soil nutrient balance through the application of organically extracted seaweed solutions. Seaweed (*Gracilaria edulis*) was extracted using organic solvents-fermented buttermilk and cow urine and compared with a conventional water-based extract. Two field trials were conducted using these organic seaweed extracts to assess their effect on black gram performance. Results showed that prepared organic extracts of cow urine and fermented buttermilk were superior to conventional water extracts. Black gram yield increased by 11.9 % and 7.7 % with cow urine and fermented buttermilk extracts, respectively, compared to the water extract. Plant and grain quality indicators - including relative leaf water content (89.8 %) chlorophyll a (1.81 mg/g), chlorophyll b (0.80 mg/g), total chlorophyll, carotenoids (2.61 mg/g) Quality of black gram plants and grain such as relative leaf water content (89.8 %), chlorophyll a, (1.81) b, total chlorophyll (0.80) and carotenoids (2.61), grain protein (27.7 %) and ash were the highest with 15 % cow urine extract application. Nutrient uptake by black gram grain and stover was also the highest with 15 % cow urine extracts application. Therefore, the study recommends applying cow urine-based seaweed extracts at a concentration of 10-15 % to optimize black gram growth and yield.

Keywords: black gram; extraction; nutrient; organic; seaweed; yield

Introduction

The agricultural sector currently faces the dual challenge of increasing food production to support a growing population while maximizing resource use efficiency and minimizing adverse effects on the environment and human health (1). To support the growing population while producing a good amount of food and maintaining the environment, biostimulants are a good contender to achieve these goals. There are many types of biostimulants, out of which seaweed extract is the most popular and trusted (2). Seaweed extract's impact on the growth and yield of crops has been studied by many researchers and has been concluded to increase the yield (3, 4). It also improves soil remediation, water retention and microbiology, while also providing nutrients and hormones for the plants (5). However, the promotion of growth by seaweed extracts depends on the concentration of application (6). Foliar spray of *Kappaphycus* and *Gracilaria* extracts at a 10 % concentration has been reported to significantly improve black gram yield (7). Several other studies have corroborated these findings, highlighting the potential of seaweed extracts to contribute meaningfully to sustainable food systems (8-10).

Seaweed has been extracted by various methods into different forms, including Liquid Seaweed Fertilizer (LSF), Seaweed Liquid Fertilizer (SLF), Liquid Fertilizer (LF) either uncut or powdered, all of which have been shown to be beneficial for cereals, pulses and flowering plants (11). Most seaweed extracts were conventionally prepared by using water as primary solvents. Other solvents such as methanol, ethanol and acetone have been used; however, these are not suitable for organic agriculture due to their synthetic nature. Therefore, there is a pressing need to develop seaweed extraction methods using organic and on-farm accessible solvents that align with the principles of organic farming.

Black gram (*Vigna mungo* L.) is an important pulse crop widely grown in India. It is highly consumed as curry, dosa, idli and many other dishes. Black gram seed contains numerous bioactive compounds such as carotenoids, tocopherol, tocotrienol, polyphenols, phytosterols, enzyme inhibitors, phytic acid, lectins, saponins and non-digestible carbohydrates that plays an important role in human physiology and metabolomics. Moreover, these metabolites have anticancer, antioxidant and anti-inflammatory properties (12). Despite its nutritional content, cultivation of black gram in India is done in Rice fallow without nutrient supplements. Thus, nutritional supplements that improve growth and yield without exhausting the soil condition must be evolved. In this context, the present study was undertaken to evaluate the effects of various organic seaweed extracts on the growth, yield and quality of black gram.

Materials and Methods

Collection and preparation of organic extracts of seaweed

Seaweed species *Gracilaria edulis* was collected from Mandapam, Tamil Nadu, India. The seaweed was washed with sea water to remove dirt and debris. After it was brought to Nammazhvar Organic Farming Research Centre, Coimbatore, India. The seaweed was again washed with tap water and distilled water and then air dried. Air dried seaweed was oven dried at 60 ± 5 °C and powdered with a Willy mill. The powdered seaweed was extracted using different organic solvents such as fermented buttermilk, cow urine and water. Fermentation of milk to obtain buttermilk was done using commercial yeast at the rate of 30 g/L for 24 hr. The curd obtained was churned thoroughly to break down the lumps and then allowed to ferment for another 48 hr in room temperature, followed by dilution with distilled water in the ratio 1:5 and then filtered. Fresh cow urine was used for extraction of seaweed in the experiment. Both fermented buttermilk and cow urine were selected as extraction solvent of seaweed due to its bioactive compounds and nutrients composition, respectively. Extraction of seaweed using different organic solvents was done according to the procedure given in previous studies (13).

Mineral content analysis

The mineral content of the raw seaweed, organic extracts of seaweed, black gram grain and stover were estimated by the methods described by Association of Official Analytical Chemists (14). The total nitrogen (N) content was analysed by micro-Kjeldahl method using the Kelplus Classic-DX VA instrument. The total phosphorus (P) concentration was analysed using Double beam Shimadzhu (Model 1800) UV-VIS Spectrophotometer. Total potassium and sodium concentrations were analysed using flame photometer (Elico). The secondary (Ca, Mg, S) and micro-nutrients (B, Fe and Cu) contents were analysed using Thermo Fischer ICP-OES 7000.

Details of field experiments

Field experiments on the effect of organic extracts of seaweed on black gram were carried out in two locations during February-May, 2024. The field experiments were carried out in factorial randomized block design (FRBD) with three replicates. The first experimental location was at Wetland Farm, Tamil Nadu Agricultural University, Coimbatore, India (11.003′N latitude, 76.92′E longitude) and the second location was at Isha Farm, Pemmelu, India (10.99′N latitude, 76.75′E longitude). Two experimental factors were considered: Factor A included the types of extraction solvents (E) used to prepare the seaweed extracts and Factor B included the different concentrations of extract applied (C). Treatments were administered at the vegetative stages (30 days after sowing, DAS) and the pod development stage (50 DAS). No major pest or diseases incidence was observed during the experiment. Details of the treatments and prevailing weather conditions during the experimental period are presented in Table 1 and Fig. 1, respectively.

Black gram (var Vamban 11) was line sown in the experimental plot (4 × 4 m^2) maintaining the row spacing and plant spacing at 30 and 10 cm, respectively. Vermicompost was applied during land preparation at the rate 12 tonnes ha^{-1}. Three hand weedings was done at 25, 45 and 63 DAS. Three irrigations were given at 27, 48 and 65 DAS. Foliar spray of the organic extracts of seaweed was done with a hand sprayer in each plot according to treatments.

Seed yield, stover yield and harvest index calculation

After threshing, the seed was collected from each plot and was used for calculating seed yield (kg ha^{-1}), the remaining plant parts were recorded for stover yield. The harvest index was calculated with the following formula:

$$\text{Harvest index (\%)} = \frac{\text{Economic yield kg ha}^{-1}}{\text{Biological yield kg ha}^{-1}} \times 100 \quad (1)$$

Grain/stover nutrient content and uptake

Major nutrient (N, P and K) content of grain and stover was converted to kg ha^{-1} using to the following formula given by (15):

Nutrient content in grain (kg ha^{-1}) =

Grain yield (kg ha^{-1})oven dry basis × Nutrient content (%) ÷ 100 (2)

Nutrient content in stover (kg ha^{-1}) =

Stover yield (kg ha^{-1})oven dry basis × Nutrient content (%) ÷ (3)

Nutrient content in grain (g ha^{-1}) =

Grain yield (g ha^{-1})oven dry basis × Nutrient content (%) ÷ 100 (4)

Nutrient content in stover (g ha^{-1}) =

Stover yield (g ha^{-1})oven dry basis × Nutrient content (%) ÷ 100 (5)

Secondary nutrient (Ca, Mg and S) and micro-nutrient (Fe, Zn, B, Mn and Cu) contents were converted to g ha^{-1} using the following formula:

Table 1. Treatment details of the field experiment

Abbreviations	Treatment descriptions
Factor A: Extraction solvents (E)	
E_1	Water
E_2	Fermented buttermilk
E_3	Cow urine
Factor B: Concentration levels (C)	
C_1	5 %
C_2	7.5 %
C_3	10 %
C_4	12.5 %
C_5	15 %

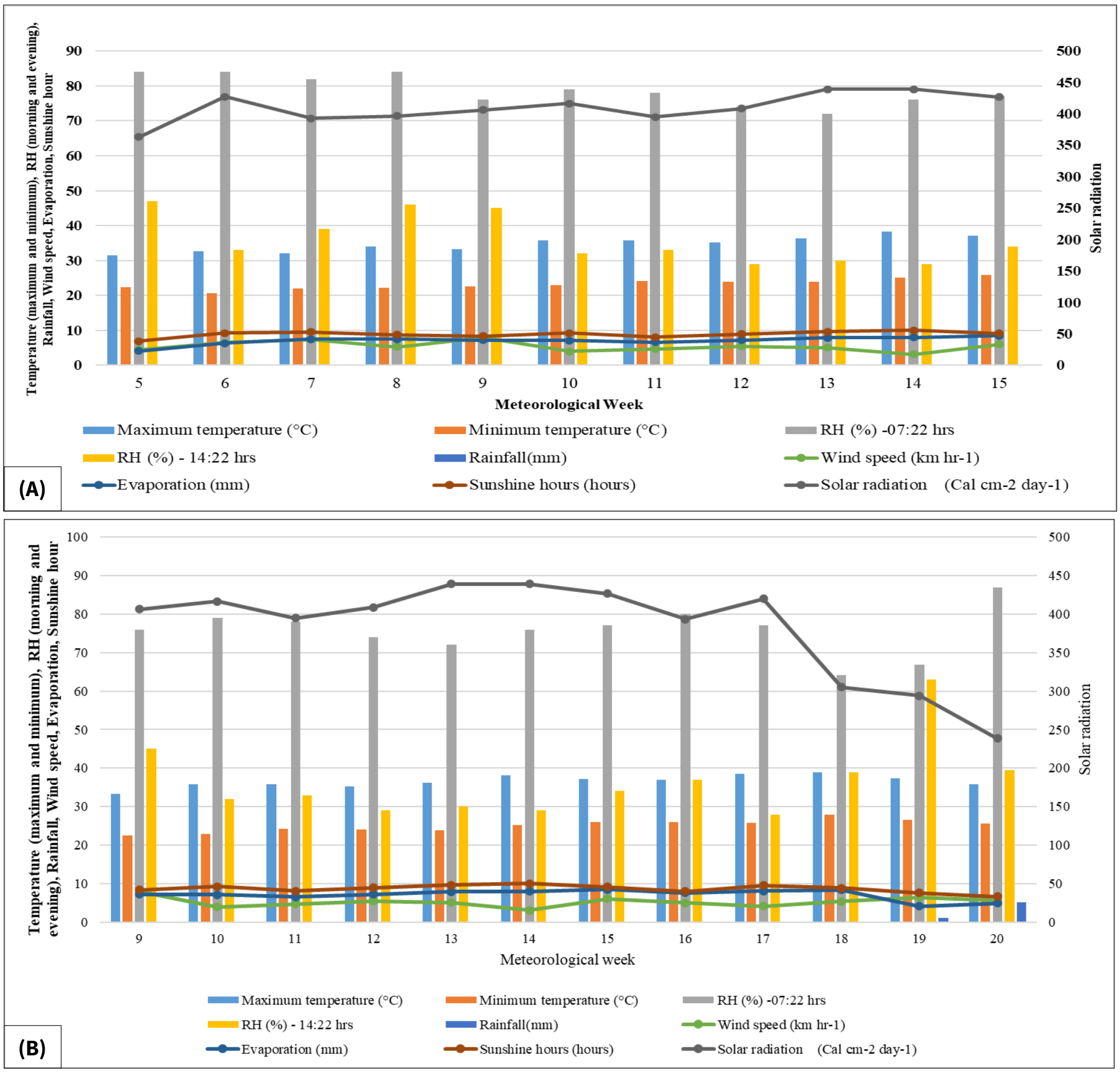


Fig. 1. Meteorological data during the field experiments. (A) Location 1, (B) Location 2.

$$\text{RLWC (\%)} = \frac{F_W - D_W}{T_W - D_W} \times 100 \quad (6)$$

Estimation of quality parameters of black gram

Relative Leaf Water content (%): RLWC content of black gram was estimated according to the methods described (16) and was expressed using the formula:

$$\text{Chlorophyll a} = (12.7 \times A663) - (2.69 \times A645) \frac{V}{W} \times 1000 \quad (7)$$

$$\text{Chlorophyll b} = (22.9 \times A665) - (4.68 \times A663) \frac{V}{W} \times 1000 \quad (8)$$

$$\text{Total chlorophyll} = (20.2 \times A645) + (8.02 \times A663) \times \frac{V}{W} \times 1000 \quad (9)$$

where, F_W is fresh weight of the leaf sample,

$$\text{Cartenoid} = (7.6 \times A480) - (1.49 \times A510) \times \frac{V}{W} \times 1000 \quad (10)$$

D_W is oven dried weight of the leaf samples and T_W is the turgid weight of the leaf sample.

Proline content: Proline content was estimated at 40 DAS according to methods described (17).

Chlorophyll content: Black gram leaf chlorophyll content was measured at 40 DAS according to the methods in the previous studies (18) and was calculated according to the given formulas:

$$\text{Cell membrane stability (\%)} = \left[1 - \frac{C_1}{C_2}\right] \times 100 \quad (11)$$

Cell membrane stability: Cell membrane stability of black gram leaf was estimated according to (19) and was calculated according to the following formula:

where, C_1 was the initial electrical conductivity of leaf leachates and C_2 was the final electrical conductivity of leaf leachates.

Statistical analysis

The Factorial Randomized Block Design (FRBD) analysis of the experiment was done in Excel 2013. The correction and graphs were analysed and extracted using OriginPro 2024b.

Results

Nutritional composition of organic extracts of seaweed

Mineral composition of seaweed and its organic extracts are given in Table 2. Among the organic extracts of *Gracilaria edulis*, the highest N (791 mg/100g), P (121 mg/100g), K (6337 mg/100g), Mg (115.6 mg/100g), S (11.7 mg/100g) and Fe (5.45 mg/100g) was observed in cow urine extracts, whereas the highest Zn (0.77 mg/100g), Mn (4.78 mg/100g) and Cu (0.35 mg/100g) was observed in fermented buttermilk extracts. The highest Ca (37.37 mg/100g) and Na (0.20 mg/100g) content was

Table 2. Mineral composition of organic solvents, seaweeds and its extracts

Mineral content	*Gracilaria edulis*	Water extract	Fermented buttermilk extract	Cow urine extract
N (mg/100g)	196.00 ± 59.40	84.00 ± 39.60	42.00 ± 19.80	791.00 ± 198.09
P (mg/100g)	95.96 ± 4.24	21.91 ± 4.51	43.19 ± 18.05	121.79 ± 70.52
K (mg/100g)	10288.0 ± 633.57	1725.81 ± 331.19	3880.00 ± 314.68	6337.50 ± 791.50
Ca (mg/100g)	337.84 ± 21.93	37.27 ± 3.22	27.31 ± 1.66	18.34 ± 4.08
Mg (mg/100g)	106.61 ± 12.68	87.96 ± 7.95	79.55 ± 4.59	115.60 ± 10.07
S (mg/100g)	2.21 ± 0.12	1.86 ± 0.11	2.3 ± 0.20	11.7 ± 0.14
Na (mg/100g)	0.31 ± 0.03	0.20 ± 0.05	0.05 ± 0.01	0.13 ± 0.02
Fe (mg/100g)	6.51 ± 37.43	2.77 ± 1.69	3.63 ± 0.59	5.45 ± 1.08
Zn (mg/100g)	3.22 ± 2.05	0.40 ± 0.01	0.77 ± 0.06	0.13 ± 0.03
Mn (mg/100g)	65.35 ± 3.13	1.26 ± 0.23	4.78 ± 0.27	0.03 ± 0.01
Cu (mg/100g)	5.66 ± 0.18	0.04 ± 0.04	0.35 ± 0.07	0.11 ± 0.03

observed in water extract.

Comparing the organic extracts and the raw seaweed of *Gracilaria edulis*, major nutrients such as N and P, secondary nutrients such as Mg and S and micro-nutrients such as Fe increased when extracted with cow urine. However, the extraction efficiency for elements like Ca, Na, Zn and Cu was lower across the organic solvents, indicating that these nutrients were less readily solubilized by organic media.

Organic extracts of seaweed on phenophase and growth attributes of black gram

The phenological stages of black gram varied depending on the type of organic seaweed extract applied (Table 3). Crops treated with cow urine and fermented buttermilk extracts of *Gracilaria edulis* completed its life cycle in 73 and 74 days, respectively. On the other hand, crops sprayed with water extract completed their life cycle in 75 days. The same trend was also observed for other phenological stages such as flowering and pod initiation with cow urine at 32 and 39 days and at 33 and 40 days with water extract, respectively. This might be due to higher stress induced by cow urine and fermented buttermilk extract treated plants forcing the plant to shorten the vegetative stage. Among the concentration levels, reduction in number of days to different phenological phases was observed with increase in concentration of the organic extracts of seaweed. At 15 % (C_5), flowering, pod initiation and maturity were observed at 32, 39 and 73 days, respectively, however, at 2.5 % (C_1) foliar spray, flowering, pod initiation and maturity were observed at 33, 41 and 75 days, respectively.

During the initial growth stages, black gram showed enhanced development in plant height, leaf area index (LAI), dry matter accumulation, crop growth rate (CGR) and net assimilation rate (NAR), irrespective of the extraction solvent used (Fig. 2). Both CGR and NAR followed a gradually decreasing trend from flowering towards pod developmental stages during both years. NAR of fermented buttermilk extract treated plants were comparatively higher than solvents. Maximum plant height, dry matter accumulation plant^{-1} and LAI of 41.3 cm, 19.77 g plant^{-1} and 3.56 m^2 m^{-2} were observed from fermented buttermilk extracts. Regarding concentration levels, 15 % foliar spray consistently led to the highest growth metrics, while the lowest values were observed at the 2.5 % concentration.

Quality parameters of black gram plant and grain

Significant variation in relative leaf water content (RLWC) of black gram was observed across different extraction solvents and concentration levels (Table 4). Among the extraction solvents, RLWC was highest in cow urine extracts and lowest in water extracts. Among the concentration levels, 15 % observed highest RLWC, followed by 10 % and lowest with 2.5 %. Proline content maximizes in cow urine extract (4.55 μ mol g^{-1} fw) and at 15 % foliar spray (4.67 μ mol g^{-1} fw). Chlorophyll a, b, total chlorophyll and carotenoids content of black gram leaves was observed to be higher in cow urine extracts (1.80, 0.81, 2.61 and 0.40 mg g^{-1} fw) and lower in water extract among the extraction solvents. Increase in chlorophyll content of black gram with cow urine extract might be due to higher nutrient content of the extract such as nitrogen, potassium and calcium that might have helped increase the chlorophyll production (20).

Table 3. Pooled data for phenophase stages and growth parameters of black gram effected by organic extracts of seaweed

Treatments	Phenophase stages (No. of days)			Growth parameters					
	Flowering	Pod initiation	Maturity	Plant height (cm)	Dry matter (g plant^{-1})	No. of root nodule plant^{-1}	LAI (m^2 m^{-2})	CGR (g m^{-2} day^{-1})	NAR (g m^{-2} leaf area day^{-1})
Extraction solvents (E)									
E_1	33.62	40.88	75.48	37.25	16.12	29.79	2.78	7.86	0.771
E_2	33.25	39.82	74.74	43.42	19.77	33.14	3.56	7.42	0.803
E_3	32.28	39.53	73.77	41.30	18.45	35.83	2.98	7.74	0.809
SEm ±	0.08	0.05	0.11	0.27	0.11	0.06	0.08	0.05	0.006
CD (*p* = 0.05)	0.22	0.16	0.32	0.80	0.32	0.18	0.25	0.14	0.008
Concentration levels (C)									
C_1	33.47	41.17	75.18	38.15	16.55	32.56	2.88	7.78	0.774
C_2	33.29	40.57	74.99	39.42	17.54	32.67	2.92	7.72	0.784
C_3	33.16	40.10	74.86	40.85	18.18	32.87	3.01	7.65	0.796
C_4	32.77	39.53	74.48	42.03	18.93	33.16	3.32	7.66	0.803
C_5	32.56	39.01	73.82	42.81	19.36	33.35	3.41	7.57	0.814
SEm ±	0.07	0.10	0.14	0.36	0.14	0.11	0.11	0.07	0.008
CD (*p* = 0.05)	0.19	0.32	0.41	1.03	0.41	0.31	0.32	0.20	0.018
Interactions (E × C)									
SEm ±	0.11	0.12	0.14	0.61	0.24	0.32	0.19	0.16	0.009
CD (*p* = 0.05)	0.33	0.43	0.51	1.79	0.71	0.93	0.56	0.47	0.034

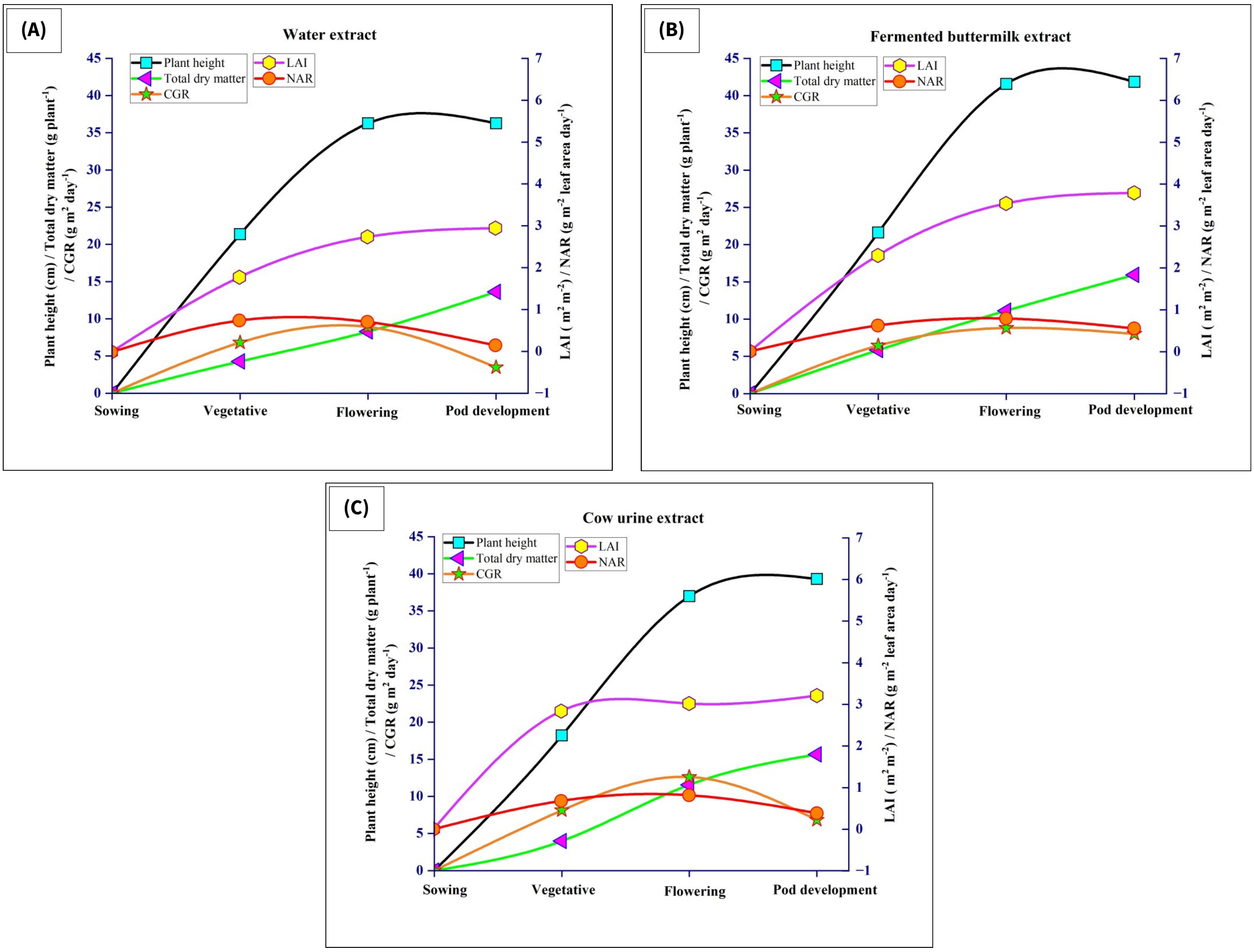


Fig. 2. Growth pattern of black gram as effect by foliar spray of organic seaweed extracts. (A) Growth pattern of black gram treated with water extract, (B) Growth pattern of black gram treated with fermented buttermilk extract, (C) Growth pattern of black gram treated with cow urine extract.

Table 4. Pooled data of black gram plant and grain quality as influence by organic extracts of seaweed

Treatments	RLWC 40 DAS (%)	Proline content (µ mol g^{-1} fw)	Cell membrane stability (%)	Chlorophyll a (mg g^{-1} fw)	Chlorophyll b (mg g^{-1} fw)	Total chlorophyll (mg g^{-1} fw)	Caratenoids	Protein content of grain (%)	Grain ash (%)
Extraction solvents (E)									
E_1	87.95	3.73	45.86	1.42	0.72	2.15	0.31	27.05	4.74
E_2	88.61	4.24	52.70	1.63	0.78	2.42	0.36	27.40	4.83
E_3	89.80	4.55	55.04	1.80	0.81	2.61	0.40	27.70	4.99
SEm ±	0.12	0.08	0.62	0.03	0.01	0.04	0.01	0.15	0.07
CD ($p = 0.05$)	0.34	0.24	1.79	0.09	0.04	0.10	0.04	0.42	0.19
Concentration levels (C)									
C_1	87.98	3.69	46.63	1.47	0.71	2.18	0.29	26.71	4.61
C_2	88.50	3.89	48.54	1.54	0.73	2.27	0.32	27.11	4.75
C_3	88.85	4.19	50.89	1.62	0.77	2.39	0.36	27.37	4.81
C_4	89.11	4.41	53.89	1.70	0.81	2.51	0.39	27.72	4.94
C_5	89.47	4.67	56.03	1.76	0.82	2.59	0.41	27.99	5.15
SEm ±	0.15	0.11	0.80	0.04	0.02	0.04	0.02	0.19	0.09
CD ($p = 0.05$)	0.44	0.32	2.31	0.11	0.04	0.13	0.05	0.54	0.25
Interactions (E × C)									
SEm ±	0.26	0.19	1.18	0.07	0.02	0.07	0.03	0.32	0.15
CD ($p = 0.05$)	0.77	0.56	4.00	0.20	0.07	0.22	0.08	0.94	0.43

Among the extraction solvents, the highest protein and ash contents in black gram grains were recorded in the cow urine extract treatment, with values of 27.70 % and 4.99 %, respectively. With respect to concentration levels, the 15 % foliar spray resulted in the highest grain protein (27.99 %) and ash (5.15 %) contents.

Yield and yield attributes of black gram

The number of pods per plant and seed yield were maximized with foliar application of cow urine extract among the different solvents and at the 15 % concentration level (Table 5). However, stover yield of fermented buttermilk (1132.51 kg/ha) and cow urine extract (1035.41 kg/ha) treat plant were statistically equivalent with each other and water extract significantly has less stover yield. Due to increase in stover yield of fermented buttermilk extract, reduction in harvest index (41.98 %) was observed even though higher yield was observed. Test weight was observed to be statistically non-significant irrespective of extraction solvents and concentration levels. The highest benefit-cost (B:C) ratio was recorded with cow urine extract (1.66) and at 15 % concentration (1.69), indicating improved economic returns.

Nutrient uptake of black gram grain and stover

The pooled nutrient uptake of black gram grain and stover as influence by organic seaweed extract are depicted in Fig. 3. Overall nutrient uptake of the stover was higher in fermented buttermilk extract treated plants compared to cow urine extract and water extract which reduces with decrease in concentration of the organic extracts. The highest N (36.47 kg/ha), P (3.97 kg/ha), K (8.13 kg/ha) uptake of black gram grain was with cow urine extracts and the lowest with water extract (N-31.57 kg/ha, P-3.09 kg/ha and K-6.57 kg/ha). However, black gram stover major nutrient content was higher in fermented buttermilk extract (N-49.0 kg/ha and K-36.27 kg/ha) than other organic extracts, except for potassium that was the highest in cow urine extract (8.93 kg/ha). Across all extracts, the 15 % concentration consistently resulted in the highest nutrient uptake in both grain and stover.

Correlation of organic extracts and black gram growth

The correlation analysis between the total nutrient content of the organic extracts and plant growth parameters revealed a positive relationship overall (Fig. 4). The maximum correlation was observed in the number of root nodules plant^{1} (0.63, 0.70 and 0.73 with cow urine extract, water extract and fermented buttermilk extract, respectively). Other plant growth parameters such as plant height (0.032, 0.076 and 0.13 with cow urine extract, water extract and fermented buttermilk extract, respectively) and dry matter accumulation (0.023, 0.070 and 0.13 with cow urine extract, water extract and fermented buttermilk extract, respectively) have a positive correlation with the nutrient content of the extracts, however, LAI (-0.26, -0.23 and -0.17 with cow urine extract, water extract and fermented buttermilk extract, respectively) showed a negative correlation corresponding to the nutrient content of the organic extracts. Fermented buttermilk extracts had the highest correlation irrespective of organic extracts and growth parameters.

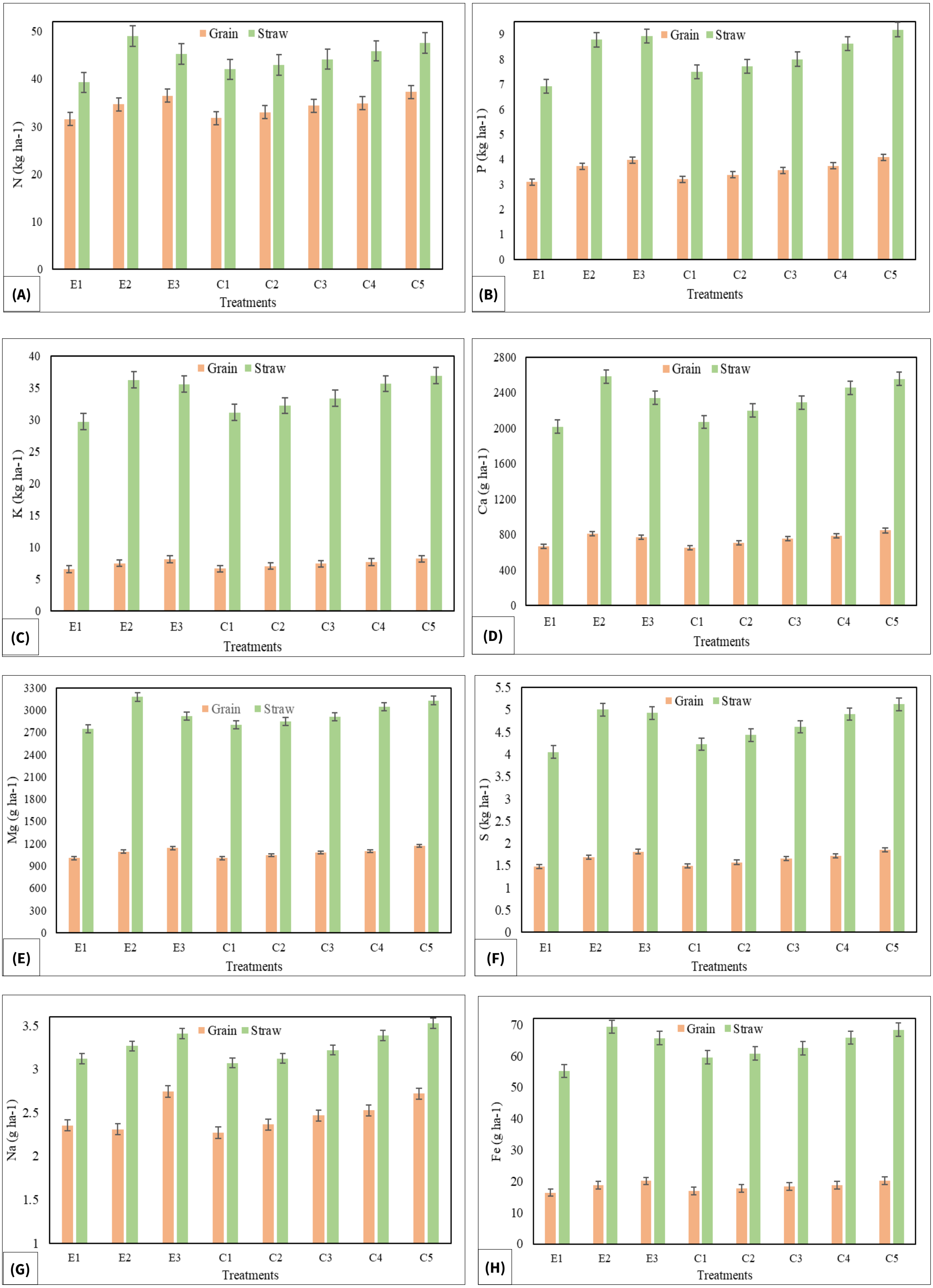
Discussion

The use of synthetic fertilizers, herbicides and insecticides is harmful to soil ecology and makes it unfit for crop growth. Moreover, intensive farming which is highly practiced for crop cultivation in India depletes soil nutrients. Due to such soil depletion, soil salinity, high calcium carbonate concentration and high or low pH levels occur (21, 22). This leads to changes in the interaction of soil micro- and macronutrients that impact the nutrient availability to plant (23). To solve the situation faced by synthetic inputs, the use of natural biostimulants in crops is a novel method. Biostimulants improve soil sustainability and plant productivity while being environmentally benign, among which seaweed gains the most attention (24). Seaweed contains many compounds that can improve plant growth. It improves soil remediation, water retention and microbiology, while also providing nutrients and hormones for the plants. The effectiveness of seaweed extracts in promoting plant growth is often dependent on the concentration applied (25).

Compared to the findings of past studies, the nutrient composition of *Gracilaria edulis* extracts in the present study was considerably higher. For instance, potassium content in the cow urine extract was 63.4 mg/g, nearly 10 times greater than the 0.7 mg/g was reported (26). Furthermore, the absence of phosphorus was reported in *Gracilaria edulis* sap, whereas, the present study recorded P levels ranging from 21 to 121 mg/100g (Table 2). In *Gracilaria edulis* organic extracts of the present study, secondary nutrient content such as Ca content was higher in water extract and lower values were observed in fermented buttermilk and cow urine extracts compared to

Table 5. Pooled yield and yield attributes of black gram as influence by organic extracts of seaweed

Treatments	No. of pods plant^{-1}	Test weight (g 100 seeds)	Seed yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Harvest index (%)	B:C ratio
Extraction solvents (E)						
E_1	35.97	6.33	769.17	986.82	43.61	1.48
E_2	38.74	6.35	828.88	1132.51	41.98	1.59
E_3	40.49	6.37	861.18	1035.41	44.47	1.66
SEm ±	0.09	0.01	4.30	2.42	0.05	-
CD (p = 0.05)	0.22	NS	12.46	7.04	0.14	-
Concentration levels (C)						
C_1	34.12	6.12	768.24	1011.92	42.66	1.48
C_2	35.39	6.24	795.21	1019.45	42.91	1.53
C_3	38.89	6.26	821.38	1038.85	43.35	1.58
C_4	41.24	6.29	831.23	1078.28	43.65	1.60
C_5	42.37	6.30	882.66	1109.39	44.18	1.69
SEm ±	0.12	0.01	7.17	4.05	0.08	-
CD (p = 0.05)	0.36	NS	20.77	11.73	0.23	-
Interactions (E × C)						
SEm ±	0.37	0.03	21.51	12.14	0.24	-
CD (p = 0.05)	1.06	0.08	62.30	35.18	0.70	-


(A)
N (kg ha-1)
Grain
Straw
E1 E2 E3 C1 C2 C3 C4 C5
Treatments
(B)
P (kg ha-1)
(C)
K (kg ha-1)
(D)
Ca (g ha-1)
(E)
Mg (g ha-1)
(F)
S (kg ha-1)
(G)
Na (g ha-1)
(H)
Fe (g ha-1)

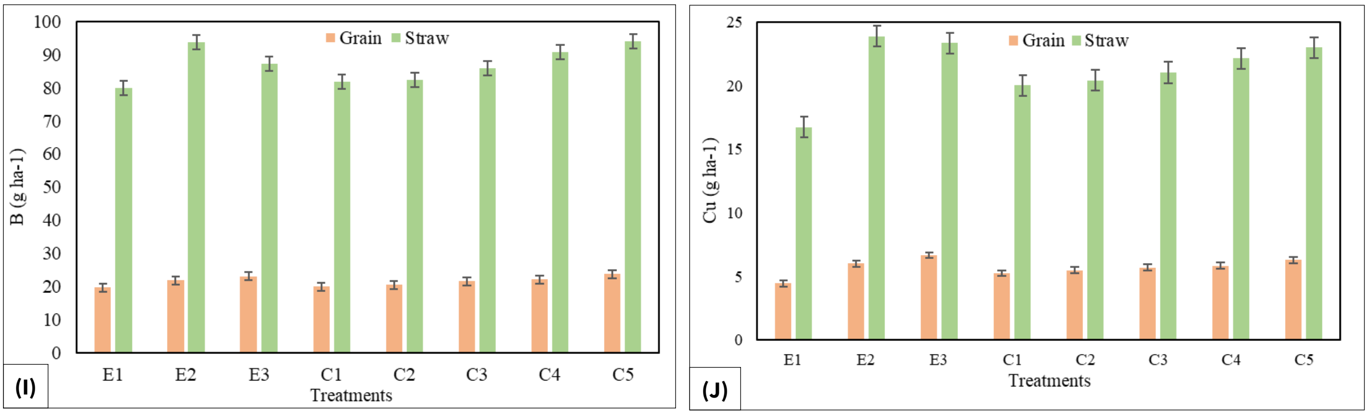


Fig. 3. Pooled nutrient uptake of black gram grain and stover as affected by foliar spray of organic extracts of seaweed. (A) Nitrogen uptake, (B) Phosphorus uptake, (C) Potasium uptake, (D) Calcium uptake, (E) Magnesium uptake, (F) Sulphur uptake, (G) Sodium uptake, (H) Irone uptake, (I) Boron uptake, (J) Copper uptake.

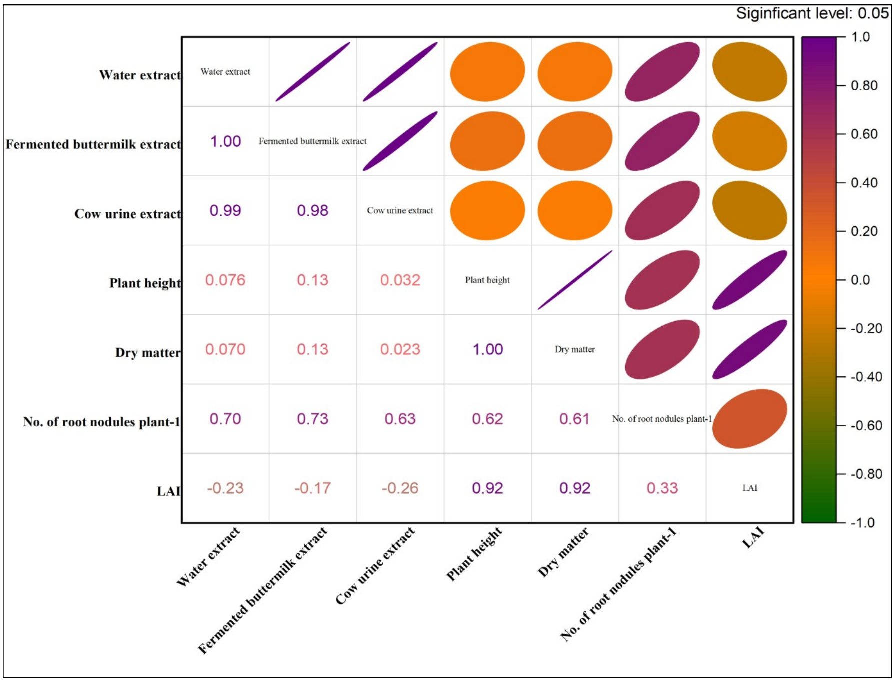


Fig. 4. Correlation matrix of organic extracts of seaweed and black gram growth parameters.

secondary nutrients reported in previous studies (26), Ca (352 mg/L), Mg (311 mg/L) and Cu (0.04 mg/L). They also reported that Zn (0.63 mg/L), Mn (33 mg/L) and Fe (13 mg/L) were present among micronutrients and Cr (0.02 mg/L) and Ni (0.21 mg/L) in heavy metals. The Mg and Cu content was higher in the present compared to the content reported (26). Notably, Mn levels were higher in the fermented buttermilk extract but lower in the water and cow urine extracts. The variation in nutrient composition could be attributed to differences in extraction methods, solvent properties and seaweed source material. Use of organic solvents might have enhanced the seaweed extracts nutrient content as observed with the increase in the nutrient content of the raw seaweed and the organically seaweed extracts especially with cow urine (Table 2). With fermented buttermilk, there might be changes in the microbial activity of the seaweed extract which has to be proven in further studies. However, there is no such experiment conducted with regards to extraction of seaweed using organic solvents, further studies have to be conducted to reveal the full potential of organic solvents extracted seaweeds.

Both field experiments were conducted in clay soil which has been used as organic farming for more than 5 years. Hence, there was little variation between the two experimental sites as they belong to the same climatic conditions. The present study clearly demonstrates that organic seaweed extracts significantly enhanced the growth and yield attributes of black gram. Improvements in plant height, dry matter accumulation, LAI, CGR and NAR collectively contributed to better seed yield and quality (Table 3). These enhancements are influenced by cultural practices and types of input used during crop growth development (27-29). With increase in growth of plants, root growth has been enhanced with a greater number of root nodules. Root nodules directly impact the number of pods per plant ultimately affecting the yield. The root nodules of black gram in the present study increase with application of cow urine and fermented buttermilk extract compared to water extract. This might be due to higher nutritional composition of the extracts affecting the root formation directly. The growth trends (Fig. 2) indicate that although all treatments started with similar initial growth, the rate of increase differed, with fermented buttermilk showing the greatest stimulation, followed by cow urine extract.

The increased in yield of black gram when treated with cow urine extract might be due to nutritional composition of cow urine in addition to the nutrient composition of seaweed. Reduction in yield of fermented buttermilk extract compared to cow urine extracts might be due to exhaustion of the nutrient of the vegetative growth of the plants resulting in less nutrients available of the pod formation. With fermented buttermilk extract, an increase in growth was observed in black gram (Table 5). This might be due to microbial composition of fermented buttermilk that enhanced the vegetative growth of the plant. Fermented buttermilk when applied alone enhance growth of plants specifically on wheat (30). In the present study, combination of fermented buttermilk with seaweed might have enhanced nutritional content that was beneficial of black gram vegetative growth. The B:C ratio clearly showed the potential profit that farmers can get through organic extracts of seaweeds such as fermented buttermilk and cow urine compared to water extract. Fermented buttermilk and water extracts have higher correlation coefficient than that of cow urine extracts, which might be attributed to the higher nutritional content of cow urine extracts and almost similar plant growth. Furthermore, this might be linked to the genetic potential of the black gram variety, which does not respond to increased levels of nutrition provided by cow urine extract. Application of cow urine and fermented buttermilk in the farm at large scale might change the microbial properties and organic matter of the soil (30, 31), however, no phytotoxicity of both cow urine and fermented buttermilk has been recorded.

Conclusion

This study demonstrates the practical potential of using easily accessible organic solvents, such as cow urine and fermented buttermilk, for extracting bioactive compounds from seaweeds. Among the tested extracts, cow urine exhibited the highest nutrient content, which translated into significantly improved growth and yield of black gram in the field conditions. The impact of the cow urine extract on the growth and yield of black gram was proved from the field experiments. Foliage (3.56 m^2 m^{-2} LAI) and vegetative growth (43.43 cm plant height) was higher in fermented buttermilk extracts, however, yield was the highest in cow urine extracts (861.18 kg ha^{-1}). These findings suggest that seaweed-based organic extracts, particularly those using cow urine, offer a sustainable, cost-effective and farmer-friendly strategy for improving black gram productivity. This approach holds promises for broader application in organic and sustainable agricultural systems.

Authors' contributions

PL carried out field experiment and prepare original draft. RK was the experiment supervisor, designed the experiment and review the manuscript. PJ designed the experiment, finalized the manuscript and formal analysis. MH carried out the statistical analysis and formal analysis. BL participated in the sequence alignment. JL participated in the sequence alignment. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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