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Utilization of seaweeds in enhancing productivity and quality of black gram [*Vigna mungo* (L.) Hepper] for sustainable agriculture

Ganesh R Jadhao[1], Doongar R Chaudhary[2*], VA Khadse[1] and Sudhakar T Zodape[2*]

[1]Department of Agronomy, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola- 444104, Maharashtra, India

[2]CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar-364002, Gujarat, India

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Seaweed and seaweed-derived products have been widely used as biostimulants for crop production, however, the biostimulatory potential of many seaweed's extract has not been fully exploited due to the lack of scientific data on field experiments. In present investigation, a field experiment was carried to study the corollary of foliar application of seaweed extract on black gram [*Vigna mungo* (L.) Hepper] under rainfed condition during rainy (*kharif*) season of 2010. Seaweed extracts (prepared from *Kappaphycus alvarezii* and *Gracilaria edulis*) were applied to the foliage at diverse concentrations (0, 2.5, 5, 10 and 15%) twice during the crop period. It was found that both the extracts were very effective and enhanced the yield, growth and improved the quality of the produce. The yield of seed had increased by 47.52% and 42.52% with the application of *K. alvarezii* and *G. edulis* extracts, respectively when applied at 10% conc. Not only this, but other yield attributing characters, viz. number of pods/plant, pod weight, seed weight/plant and test weight of seed also have been augmented. The quality of seeds was also improved under the influence of the seaweed extract treatments. The foliar application of seaweed extracts to black gram crop has significantly increased uptake of almost all the nutrients. Thus, foliar applications of seaweed extracts could be a promising option for yield enhancement and sustainable agriculture.

Keywords: Black gram, *Vigna mungo,* Hormones, Seaweed extract, Nutritional quality, Yield.

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Introduction

There is a long history of coastal people using seaweeds to fertilize soil for crop production but due to bulky in nature; these are not usually carried very far. Algal biomass has also been composted and then used for growing crops on various types of soil[1]. CSIR-CSMCRI, Bhavnagar (Gujarat) developed technology to liquefied seaweed as a potential plant nutrient and the residue can be utilized for preparation of carrageenan[2]. Numerous studies have revealed a wide range of beneficial effects of seaweed extract applications on plants, such as early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress and enhanced postharvest shelf-life of perishable products[3-6]. Seaweed extracts contain major and minor nutrients, amino acids, vitamins and growth substances like cytokinins, auxins and abscisic acid (ABA)[7]. Further, seaweed extracts are considered as an organic agriculture input as they are environmentally benign and safe for the health of animals and humans[8]. Application of seaweed extract as organic biostimulant is fast becoming accepted practice in agriculture due to its beneficial effect[9,10]. Although, many of the various chemical components of seaweed extracts and their modes of action remain unknown, it is plausible that these components exhibit synergistic activity[11, 12].

Legumes are widely grown throughout the world and their dietary and economic importance is globally appreciated and recognized. Legumes not only add variety to diet but also serve as an economical source of supplementary proteins for a large human population. Grain legumes are being cultivated in India since time immemorial. They have high total protein content (20-26%) and can be considered as a natural supplement to cereals. After fish (dry) which provides 335 g protein/kg, grain legumes provide 220-250 g protein/kg. Hence, legumes are considered as a "poor man's meat". Therefore, present study was attempted to evaluate the efficacy of different concentrations of seaweed extracts (*Kappaphycus*

*Correspondent authors:
E-mail: drchaudhary@csmcri.org; stzodape@gmail.com
Tel: +91278-2569496, Fax : +91278-2567562)

alvarezii and *Gracilaria edulis*) in enhancing the growth, yield, quality and nutrient uptake of black gram [*Vigna mungo* (L.) Hepper] grown in field under rainfed condition.

Materials and Methods

Preparation of liquid seaweed extracts

The seaweed extracts were obtained from fresh *K. alvarezii* and *G. edulis* seaweeds using the methodology of Eswaran *et al*[2] and appropriate dilutions were made as per treatments along with surfactants for proper adherence for experimental purpose.

Experimental site

Field experiment was conducted during rainy (*kharif*) season of 2010 on Typic Haplustert of Vertisol at the experimental farm of Department of Agronomy, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. The soil of the site was clayey with $pH_{(1:2.5)}$ 7.8, non-saline [$EC_{(1:2.5)}$ 0.25 dS/m] with uniform and leveled topography. The experimental soil has organic carbon 0.43%, available N 178.5 kg/ha, available P_2O_5 16.4 kg/ha and available K 374.4 kg/ha. After completion of experiment, post harvest soil was analyzed for available nitrogen (N), phosphorus (P) and potassium (K), but there was no significant effect of treatments (data not shown). The climate of region is subtropical. The experimental site was located at 22º42´´ N latitude, 77º 02´´ E longitude and 307 meters above mean sea level.

Experimental design and treatments

The experiment comprised of nine treatments, viz. 0 (control), 2.5, 5, 10 and 15% (v/v) each of *K. alvarezii* and *G. edulis* extracts. Two foliar sprays of seaweed extracts were applied at 40 and 52 days after sowing of black gram. The volume of the spray was 500 L/ha in each spray. The treatments were distributed in a randomized block design with four replications. The net plot size was 3 m × 3 m.

Crop management

Field was prepared by ploughing, harrowing, stubble collection and leveling before sowing of black gram crop. The fertilizer at the rate of 20 kg N/ ha and 40 kg P_2O_5/ ha were applied using urea (46% N) and single super phosphate (16% P_2O_5), respectively at the time of sowing. Prior to sowing, the seeds were treated with Thiram followed by inoculation with *Rhizobium* and phosphate solubilizing bacterial cultures. The seeds of black gram (*Vigna mungo* var. TAU-1) were sown by drilling during July 2010 and crop was maintained under rainfed condition. Distance between row to row 30 cm and plant to plant 10 cm was maintained. The seed rate for sowing was 10 kg/ha. Plant protection measures were taken to control the diseases and pest as and when it was required. Crop was harvested at physiological maturity in the month of September, 2010.

Plant sampling

The biometric observations of plants were recorded on randomly tagged five plants, to represent the representative population in each net plot. The chlorophyll content index was measured by chlorophyll meter CCM-200 (Opti-Sciences, USA). At maturity, samples of black gram seed and straw were collected from each plot, oven dried at 70^o C to constant weight and ground to pass through a 0.5 mm sieve and used for chemical analysis. Fat content of seeds was determined by Soxhlet method using hexane as solvent. Ash content of grain was determined by ashing at 550 ^{o} C for four hours in muffle furnace. Total protein content of seeds was derived by multiplying N content with factor 6.25. The N content was determined by the semi-micro Kjeldahl method[13], after the plant tissue was oxidized and decomposed by sulphuric acid with digestion mixture (K_2SO_4: $CuSO_4$ = 5:1). Phosphorus content was determined by the vanado-molybdate yellow color method[14], K content by flame photometry[14] and Ca, Mg, S, Cu, Mn, Fe and Zn concentration by ICP-OES (Perkin Elmer, Optima 2000) after wet digestion with HNO_3-$HClO_4$ (10:4) di-acid mixture[15].

Statistical analysis

Data were analysed using analysis of variance (ANOVA) following randomized block design[16]. The significant differences among treatments were compared with the least significant difference at 5% ($p<0.05$) level of probability.

Results and Discussion

Effect of foliar spray of seaweeds extract on growth and dry matter partitioning

Foliar applications of different seaweed extract along with recommended dose of fertilizers significantly increased the growth parameters compared to control treatment (Table 1). An increase in several growth parameters such as plant height,

Table 1—Effect of seaweed extracts on growth attributing character of black gram

Treatments	Plant height (cm) (at harvest)	Root length (cm) (at harvest)	Number of leaves per plant (60 DAS)	Number of branches per plant (at harvest)	Leaf area per plant (cm^2) (60 DAS)	Nodules per plant (60 DAS)	Chlorophyll content index (60 DAS)
Control	63.8d	22.0d	9.9e	2.9d	349.8f	29.0a	33.0c
2.5% K*	68.7d	23.6cd	11.6cd	3.5c	397.1de	28.5a	38.2bc
5% K	75.9bc	24.8bc	11.9bc	3.7c	417.8cde	33.8a	41.2ab
10% K	78.8ab	26.8a	12.6ab	4.0abc	443.9abc	31.0a	45.8a
15% K	81.5a	27.7a	14.1a	4.3a	463.8a	28.8a	44.0ab
2.5% G**	72.7cd	23.2cd	10.4de	3.7c	380.8ef	28.3a	37.9bc
5% G	74.6bcd	24.8bc	11.5bcd	3.8bc	410.8cde	30.5a	38.6bc
10% G	76.8abc	26.2ab	12.4abc	3.9abc	425.8abc	31.3a	44.3ab
15% G	79.5ab	26.9a	13.6a	4.2ab	455.9ab	32.3a	42.0ab

(Different letters in a single column show statistically significant differences for P < 0.05). [*K= *Kappaphycus alvarezii*; **G= *Gracilaria edulis;* DAS=days after sowing]

Table 2—Effect of seaweed extracts on dry matter partitioning in black gram

Treatments	Leaves per plant (g) (at harvest)	Stem per plant (g) (at harvest)	Root per plant (g) (at harvest)	Pod per plant (g) (at harvest)	Dry matter per plant (g) (at harvest)
Control	2.8d	4.4d	1.2c	5.7e	14.1e
2.5% K*	3.6bcd	4.8d	1.3bc	7.2cd	16.8cd
5% K	4.3b	5.5cd	1.4bc	7.7bc	18.9c
10% K	4.4b	6.9ab	1.5abc	9.9a	22.6ab
15% K	6.3a	7.7a	1.7a	8.3b	23.9a
2.5% G**	3.1cd	4.5d	1.2c	6.7de	15.5d
5% G	4.0bc	5.5cd	1.3bc	7.3cd	18.1c
10% G	4.4b	6.2bc	1.4bc	9.6a	21.5b
15% G	6.1c	7.3ab	1.5ab	7.9bc	22.8ab

(Different letters in a single column show statistically significant differences for P < 0.05) [*K= *Kappaphycus alvarezii*; **G= *Gracilaria edulis*]

root length, number of leaves/plant, number of branches/plant, leaf area/ plant and chlorophyll content were found to be significantly higher for 15% seaweed extract concentrations (*K. alvarezii* and *G. edulis*), however, this is statistically at par with 10% concentration of seaweed extracts. The beneficial effect of seaweed extract application on growth of plants can be attributed to its components working synergistically at different concentrations[11]. Extracts of seaweeds are known to contain nutrients[17] and growth regulators such as auxins (IAA and IBA), gibberellins, cytokinins, betaines and major macro and micronutrients[5]. Increase in root length[18,19], root growth[20], number of lateral roots[21], plant height[20], number of branching[19] and chlorophyll contents[22] have been reported in different crops by seaweed extract application. The present study clearly indicates that seaweeds extracts are beneficial to enhance overall growth of black gram as compared to the control plants. Betaines and other related quaternary ammonium compounds act as anti-stressors in both biotic and abiotic stress condition[23,24]. Since, the test crop under investigation was grown under rainfed condition, at some point of time it could have faced water stress condition. Exogenous application of seaweed extract has already been shown to enhance antioxidants status of Kentucky bluegrass (*Poa pratensis* L) under water stress condition[25] and may enhanced water utilization efficiency[26].

It was found that dry weight of shoot/plant, root/plant and pods/plant significantly increased by 57, 21 and 73%, respectively over control when plants were sprayed with 10% *Kappaphycus* extract. Similarly, increase in dry weight of shoot, root and pod increased by 65, 28 and 40%, respectively over control was recorded for the plants receiving 15% *Gracilaria* extract (Table 2). This suggests that the foliar sprays of seaweed extracts have enhanced the dry matter partitioning.

Effect of foliar spray of seaweeds extract on yield and yield attributes

The effect of seaweeds extract on yield and yield attributes is presented in Table 3. An increase in yield attributes such as number of pods/plant, pod weight/ plant, grain weight/plant and test weight were found to be increased up to 10% conc. of both seaweeds extract and then declined at higher concentration (Table 3). Grain yield was significantly highest at 10% conc. of the both seaweed extracts. Compared to control, the foliar applications with 10% of *Kappaphycus* and *Gracilaria* extracts resulted into 48 and 43% increase in the seed yield, respectively over control treatment. Likewise, harvest index and grain to straw ratio were also observed to be highest in these treatments. However, maximum straw and biological yields were reported at 15% conc. of both the extracts. Cytokinins have been implicated in nutrient mobilization in vegetative plant organs[27] as well as reproductive organs[28] eventually effect the dry matter partitioning in crops. Rama Rao[29] reported increased yield and improvement in quality of *Zizyphus mauritiana* Lam. with foliar application of seaweed extract. Studies have also shown that use of seaweed extract increased fruit yield of tomato with superior quality[20], bean yield by 24% and enhanced the yield of grape[6, 30].

Effect of foliar spray of seaweeds extract on the uptake of nutrients

The use of seaweed extracts significantly improved the nutrient uptake by seed and straw of black gram (Fig. 1). It was found that the foliar application has significantly increased the uptake of almost all the nutrients under the influence of 10% of both the seaweed extracts and then declined at 15% concentration. The foliar spray of 10% of *Kappaphycus* extract exhibited percent increase in uptake of nutrients in grain in range of 62.62% (Fe) to 96.28 % (S) as compared to control. Similarly, the uptake of different nutrients in grain was found between 57.54% (Fe) to 96.81% (S) when the plants were treated with *Gracilaria* extract at 10%. In case of straw, significantly highest uptake was exhibited at 15% conc. of *Kappaphycus* extract and it ranged between 50.40% (Mn) to 64.24% (P). Nevertheless, definite trend was not found for *Gracilaria* extract. Our results corroborate with Crouch *et al* [31] who reported increased uptake of Mg, K and Ca in lettuce. Turan and Köse[32] also observed increased uptake of N, P, K and Mg in grapevine with application of seaweed extract. In addition, Nelson and van Staden[33] and Mancuso *et al*[34] had similar findings. The present study showing increment of uptake of nutrients by black gram is in conformity with the findings of Frankenberger and Arshad[35] who observed that bio-stimulants have enhanced the effectiveness of fertilizers.

Effect of foliar spray of seaweeds extract on grain quality of black gram

The analysis of various major and minor nutrients along with protein contents was carried out and results are presented in Table 4. Significant increase in major nutrients (P, K, S, Ca and Mg) as compared to control was found to be maximum for the plants treated with 10% conc. of both the seaweeds extract. However, there was no significant effect observed on N and micronutrient contents, though there was an improvement in contents of these nutrients in seeds of black gram. Protein content was increased by 15 and 17% with foliar application of 10% of *Kappaphycus*

Table 3—Effect of seaweed extracts on yield attributing character and yield of black gram

Treatments	No. of pods plant^{-1}	Pod wt plant^{-1} (g)	Grain wt plant^{-1} (g)	Test wt (g)	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})	Biological yield (q ha^{-1})	Harvest index (%)	Grain to straw ratio
Control	10.5e	5.7e	3.0e	50.8e	10.1d	28.3g	38.3e	26.2cd	0.36cd
2.5% K*	12.9d	6.7d	3.6d	51.1de	10.7cd	29.5fg	40.2e	26.8cd	0.37bcd
5% K	13.9d	7.3cd	4.1bc	51.7de	12.5b	31.8def	44.3cd	28.2bc	0.39bc
10% K	18.9a	9.9a	4.9a	53.9a	14.9a	33.6cd	48.5b	30.7a	0.44a
15% K	16.1b	8.3b	4.4b	53.4ab	13.1b	38.6a	51.7a	25.3d	0.34d
2.5% G**	13.4d	7.2cd	3.8cd	51.4de	11.2c	30.3efg	41.4de	26.9cd	0.37bcd
5% G	14.4bcd	7.7bc	4.1bc	51.7cd	12.7b	32.4de	45.1c	28.2bc	0.39bc
10% G	18.3a	9.6a	4.9a	53.8a	14.4a	35.1bc	49.5ab	29.1ab	0.41ab
15% G	15.3bc	7.9bc	4.3b	52.5bc	12.9b	37.6ab	50.5ab	25.6d	0.34d

(Different letters in a single column show statistically significant differences for $P < 0.05$) [*K= *Kappaphycus alvarezii*; **G= *Gracilaria edulis;* q=1 quintal=100 kg]

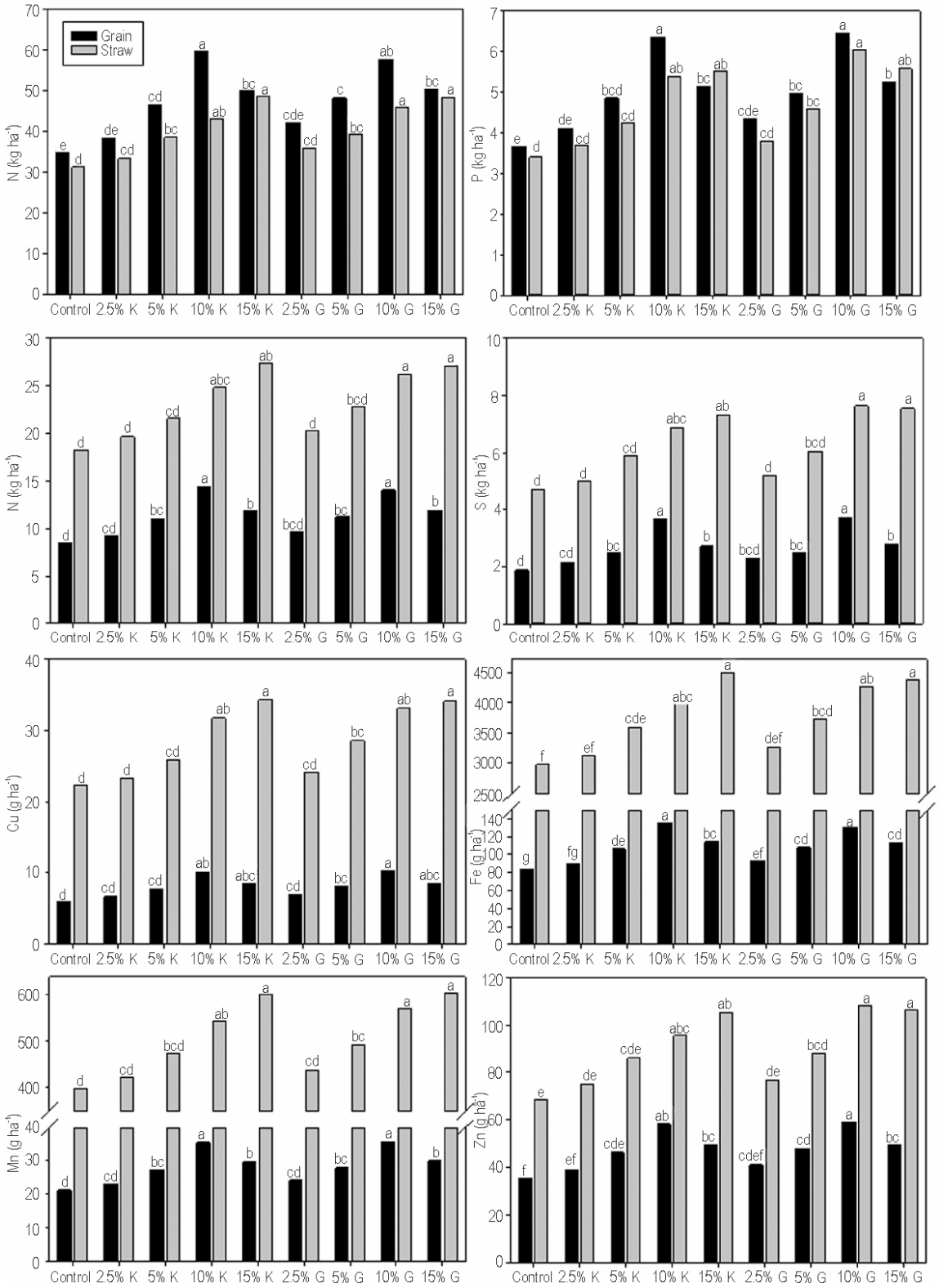

Fig. 1—Effect of seaweed extracts on nutrient uptake (grain and straw) by black gram. Similar bars with different letters are significantly different ($p<0.05$). (K= *Kappaphycus alvarezii*; G= *Gracilaria edulis*)

Table 4—Effect of seaweed extracts on nutrient contents and quality of black gram grain

Treatment	N (%)	P (%)	K (%)	S (%)	Ca (%)	Mg (%)	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Ash (%)	Crude protein (%)	Fat (%)
Control	3.49a	0.36c	0.84c	0.19c	0.10b	0.22c	5.96a	82.74a	21.13a	35.27a	4.29a	21.78a	0.99a
2.5% K*	3.57a	0.38bc	0.86bc	0.20c	0.10b	0.22c	6.16a	83.38a	21.62a	36.48a	4.31a	22.34a	1.02a
5% K	3.78a	0.39bc	0.88bc	0.20c	0.10b	0.23bc	6.23a	85.26a	21.84a	37.13a	4.32a	23.66a	1.06a
10% K	4.02a	0.42ab	0.97a	0.25ab	0.13a	0.26ab	6.70a	90.96a	23.79a	39.15a	4.39a	25.11a	1.24a
15% K	3.84a	0.39bc	0.91abc	0.21bc	0.12ab	0.23bc	6.49a	87.81a	22.64a	38.00a	4.38a	23.98a	1.22a
2.5% G**	3.77a	0.39bc	0.87bc	0.20c	0.12ab	0.22c	6.19a	83.57a	21.70a	36.72a	4.38a	23.56a	1.01a
5% G	3.81a	0.39bc	0.89bc	0.20c	0.12ab	0.24abc	6.42a	85.50a	22.01a	37.87a	4.38a	23.82a	1.13a
10% G	4.05a	0.45a	0.97a	0.26a	0.13a	0.27a	7.06a	90.99a	24.67a	40.96a	4.39a	25.29a	1.26a
15% G	3.93a	0.40bc	0.92ab	0.21bc	0.11ab	0.24abc	6.60a	87.91a	23.19a	38.53a	4.39a	24.56a	1.22a

(Different letters in a single column show statistically significant differences for $P < 0.05$).). [*K= *Kappaphycus alvarezii*; **G= *Gracilaria edulis*]

and *Gracilaria* extracts, respectively over control. Similarly, fat content in grains was also increased by 25 and 27% in *Kappaphycus* extract and *Gracilaria* extracts, respectively at 10% conc. as compared to control. The positive impact of seaweed extract on fruit quality has been reported by the Clemson research group, with peaches, vegetable crops[36], turf quality[37] and wheat quality[38].

Conclusion

In this study, experimental evidence does indicate that seaweed extracts are very effective and enhanced the yield, growth and improved the quality of the black gram. The seed yield of black gram was increased by 47.52% and 42.52% with the application of *K. alvarezii* and *G. edulis* extracts, respectively when applied at 10% conc. in comparison to control treatment. The present investigation proved that both seaweed extracts can be used as a supplemental fertilizer for conservation of natural resources, sustainable agricultural production and enhanced environmental quality.

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