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Original Article

Using brown seaweed as a biofertilizer in the crop management industry and assessing the nutrient upliftment of crops

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Abstract

Due to the increasing levels of the world population, the demand for agricultural products has also increased over the years. Enhancing the yield and nutrient content of food products is therefore an important aspect in the current context. Experiments were conducted at both lab-scale and field level with extractions from *Sargassum* sp., to assess the growth of the plant, crop production, and nutrient content of *Vigna radiata*. A lab-scale experiment was conducted to identify and distinguish the germination percentage and seedling vigor of *Sargassum* treated and non-treated seeds. This included the application of a concentration series of polysaccharides and a concentration series of crude seaweed extract. The highest germination percentage was observed at 8% polysaccharide content and 15% liquid seaweed extraction (LSE) content resulting in 14% and 8% of germination increment against the control. When increasing the polysaccharide and LSE content exceeding the above-stated values, germination rate and seedling vigor dropped. When applied as foliar applications, an increment of pods against the control showed an increment of 28%, which is the maximum increment reached the 10% LSE concentration. With the polysaccharide application, the maximum yield increment was 31% with 10% concentration. Also, the dry weight content of seeds and the number of pods per plant and seed per pod have increased considerably. Further, both polysaccharide and LSE applications have increased the micronutrient and protein content in seeds. Therefore, it is recommended to use the LSE at 15% concentration and polysaccharide at 8% concentration at the germination stage and as a foliar application to increase the yield and nutrient content of the *Vigna radiata* plant.

Keywords: Bio-fertilizer, Germination, Seaweeds, *Sargassum* sp., *Vigna radiata*

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Introduction

Organic farming is considered an essential element in agriculture and usage of chemical fertilizers has become questionable in the recent past. Therefore, many agricultural practices tend to move towards organic farming coupled with biofertilizer applications, which are contributing to the deposition of residues and thereby improving the physical and chemical properties of the soil. Among the sources of supplemental fertilizers, algal biomass has been used in various continents to different extents. Seaweeds are used as nutrient supplements and biostimulants in agricultural and horticultural crop production and are known to enhance seed germination and seedling vigor. However, the growth-promoting activities of seaweeds are yet underexploited in South Asian agriculture though many studies have highlighted the impact that the seaweeds can make on vegetables, bulbous crops, flowers, legumes, and in vitro culture conditions. Seaweed extracts are known to increase the rate of germination, root and shoot growth development, improving the quality of the fruit and resistivity to pathogens (Rayorath et al., 2008; Mattner et al., 2013; Ali et al., 2015; Singh et al., 2015). Such growth-promoting activities of plants confirm the presence of plant growth regulators such as cytokinins, gibberellins, and auxins, as well as the presence of polyamines, polysaccharides, alginates, and fucoidans in brown seaweeds (Hong et al., 2007; González et al., 2013). The growth stimulation is evident by enhancing nitrogen assimilation and basal metabolism (González et al., 2013; Zhang and Ervin, 2008; Wang et al., 2016).

One such example of growth stimulation is the application of commercial seaweed extracts of *Ascophyllum nodosum* which has improved the growth of roots in *Arabidopsis thaliana* (Rayorath et al., 2008). Apart from regulating plant growth, there are many known benefits gained from seaweeds in plant cultivations as well, including; delaying fruit senescence, improvement of yield quantity and quality, and improving the ability to withstand adverse conditions. However, without much knowledge on the species and their activity, farmers in the coastal regions of Sri Lanka, harvest seaweeds and use them directly as compost and dry matter to improve the soil condition of the coastal regions of the country. Unlike the chemical fertilizers which are famous worldwide, seaweed extracts are biodegradable, non-toxic to both animals and humans, and non-polluting (Pramanick et al., 2013; Pal et al., 2015). Henceforth, the annual production of seaweed across the globe has risen to approximately 31.2 million tons from which a higher portion is used as nutrition supplements to improve plant growth (FAO, 2018).

Though a considerable amount of studies has been conducted to study the activity of seaweed species like *Ascophyllum nodosum*, *Fucus serratus*, *Fucus vesiculosus*, and *Laminaria hyperborean*, biology, composition, and the activity as growth stimulants of *Sargassum* varieties are not been reported widely. Therefore, it is considered as an under-utilized seaweed variety. Also, *Sargassum* sp. is rich in nutrients and minerals including the plant growth regulator; cytokinin (Crouch and Van, 1993). Henceforth, this study aims to evaluate the ability to utilize *Sargassum* sp. as a biofertilizer in improving the yield of *Vigna radiata* plants in organic farming agriculture.

Material and Methods

Selection and description of the seaweed collection site

Live specimens were collected from Hikkaduwa-Sri Lanka (Latitude: 6.1313°, Longitude: 80.1007°), a straight coastline eventually with a beach rock platform. Plants were grown in condensed populations and attached to the substrate by a disc; upright branches markedly compressed, smooth and straight. Leaves are 3-4 cm long, 5-7 mm wide, upwardly directed, and alternately placed on the axis in one plane, resulting in very flat plants; Air bladders are not observed. Specimens were in a sand substratum with having a sub-horizontal general coast inclination. Seagrass and seaweed vegetation was found as the biotopes and had an ecology of submerging at low tide, sand-covered bottom under low watermark (subtidal). In this site, the *Sargassum* sp. identified have formed intricate cushions in the prostrate growth direction. The in-situ colour of the species was brown.

Seaweed sample collection from the site

Specimens of *Sargassum* sp. were detached from its substrate, cleansed and epiphyte removal was done by washing with water at the collection site itself. These samples were then placed in iced containers to facilitate transportation. After reaching the destination, samples were sun-dried for 24 hours and oven-dried at 60°C for another 48 hours. Dried samples were grounded until a fine powder was

obtained and sieved from 350 µm mesh to obtain a fine powder. The acquired powder was placed in moisture & air proof containers and stored at 4°C until further use.

Preparation of liquid seaweed extract (LSE)

As per a previously established method, (Challen and Hemingway, 1966) LSE was prepared by mixing grounded seaweed powder with distilled water in a 1:1 ratio and was allowed to stand. Then the mixture was boiled, allowed to stand for some more time, and passed through a filter paper (Whatman Grade 1: 11 µm) to remove solids. The resultant liquor was then centrifuged at 3000 rpm for 3 minutes (Kubota 4000). Solids received from the initial filtration and centrifugation were pressed and the obtained liquor was mixed with the main liquor. The resultant liquor was then concentrated under reduced pressure to obtain a brown liquid. The LSE made is stored in the refrigerated conditions at 4^0C until further use.

Preparation of polysaccharide extract

Exactly 100.00 g of the dried seaweed powder was mixed with 1 liter of distilled water and allowed to stand at 90°C for 1 hour (Makawita et al., 2019). After the designated time, the mix was cooled and centrifuged at 2000 rpm for 10 minutes. The supernatant was collected, and three volumes of absolute ethanol were added. After standing for 5 minutes, again the mixture was centrifuged, and the supernatant was removed. The resultant extracted polysaccharide was dried in the oven at 40˚C and a concentration series from 2% to 12% was prepared using distilled water (Table-2).

Preparation of the substrate for seed growth

The soil mixture was prepared by using clay, silt, and sand in 1:1:1 ratio in pots, topping up with a compost layer of ½ inch (Zodape et al., 2010). To maintain substrate uniformity, a similar method was used to prepare the medium for the control sample.

Initial preparation of *Vigna radiata* seeds

Certified beans from *Vigna radiata* MI 6 variety were purchased from the Field crop research & development institute of Mahailluppallama in Sri Lanka, where the seeds were sorted to have equal weight and size. Selected seeds were then imbibed in distilled water for 24 hours at room temperature (31°C) and drained and washed twice with distilled water and kept in dark on a filter paper surface for another 24 hours (Elke et al., 1981). Cotyledons of the seeds were then dipped in 10 ml of polysaccharide extracts and LSE concentrate series and kept for 24 hours (Table-1).

Table-1. Treatment of seeds with LSE

Sample	Condition	Duration	No. of seeds treated
A1 (Control)	Seeds soaked in distilled water	24 hours	200
A8	Seeds soaked in 10% LSE	24 hours	400
A9	Seeds soaked in 15% LSE	24 hours	400
A10	Seeds soaked in 20% LSE	24 hours	400
A11	Seeds soaked in 30% LSE	24 hours	400

Table-2. Treatment of seeds with polysaccharide extraction (PE)

Sample	Condition	Duration	No. of seeds treated
A1 (Control)	Seeds soaked in distilled water	24 hours	200
A2	Seeds soaked in 2% PE	24 hours	400
A3	Seeds soaked in 4% PE	24 hours	400
A4	Seeds soaked in 6% PE	24 hours	400
A5	Seeds soaked in 8% PE	24 hours	400
A6	Seeds soaked in 10% PE	24 hours	400
A7	Seeds soaked in 12% PE	24 hours	400

The germination percentage of seeds was calculated using the below equation, against the control sample.

$$Germination\ percentage = \frac{\text{Number of seeds with sprouts after 48 hours}}{\text{Number of seeds used for treatments}} X\ 100 \qquad \text{Eq (1)}$$

Sowing of seeds and foliar applications

The treated seeds were sown in the prepared pots and environmental conditions were given at an equal capacity from A1 to A11 samples. After the growth of root and shoot, observations were made for the first set of plants by removing from the soil substrate. For the remaining plants, foliar application of the extract was given at the flowering stage (Table-3).

Table-3. Treatments given for plants as foliar applications, at the flowering stage

Sample	Condition	Frequency	No. of plants treated
A6 (Control)	Plants sprayed with water	Twice per day (Morning 8 am, Evening 5 pm – 5 sprays each plant)	100
A12	Plants sprayed with 5% LSE		200
A13	Plants sprayed with 10% LSE		200
A14	Plants sprayed with 15% LSE		200
A15	Seeds soaked in 2% PE		200
A16	Seeds soaked in 4% PE		200
A17	Seeds soaked in 6% PE		200
A18	Seeds soaked in 8% PE		200
A19	Seeds soaked in 10% PE		200
A20	Seeds soaked in 12% PE		200

After 90 days, grain yield was calculated for all samples, along with pods per plant and grains per pod.

Analysis of micronutrient and protein content in seeds

Seeds were harvested from pods and were analyzed for micronutrients through ICP-OES and for proteins through the Kjeldahl method.

In the ICP-OES method, sample preparation was done by thoroughly blending the seeds to obtain uniformity across all samples. Homogenized samples were weighed to the nearest 0.001g into a clean microwave vessel liner. The extraction process was conducted by using microwave digestion. About 5ml of ultra-pure grade HNO_3 acid was added to each vessel and was placed in the microwave oven. After the digestion is complete after 20 minutes at 180°C, vessels were allowed to cool and were transferred to a fume-hood to attain equilibrium with the room temperature. At the evaporation stage, vessel liners were placed in evaporation carousels and were placed in the microwave oven. To reduce the final volume to 1 ml, samples were exposed to 100°C temperature for 8 minutes. Once the vessels were cooled down to the room temperature, the assembly was removed. For the vessels where the total volume was lower than 1 ml, HNO_3 was added until reaching 1 ml of volume. Then the residual digest was transferred by washing with deionized water while keeping the volume less than 35 ml. Then, 50 µl of 5000 ppm internal standard solution was added to the extract while bringing the total volume up to 50 ml with the addition of deionized water. Finally, samples and controls were placed in the analyzer to identify and calculate mineral levels.

In determining the protein content, 3 steps were involved in the process.

I. Digestion

Approximately weighed 0.05g of the specimens of the powdered *Vigna radiata* seeds were separately placed in folded papers and then in the dried Kjeldahl digestion flasks along with 2 Kjeldhal catalyst tablets ($Na_2S_2O_8$/CuSO4) and 2.5 ml of analytical reagent grade (AR) concentrated sulfuric acid (H_2SO_4, AR grade). The digestion flask was then attached to the block digestor (DK 20), and the sample was allowed to digest at 420°C for 3 hours approximately until a clear solution was obtained. Triplicates from the samples were prepared using a similar method. After completion of the digestion, 10 ml of ammonia-free distilled water was added after cooling down. Blank digestion was also carried out for each sample, without adding the sample.

II. Distillation

After the digestion was complete, contents in the digestion tubes were transferred to the distillation tube of the apparatus while adding an excess volume of 32% Sodium Hydroxide (NaOH, AR grade) and distilled water. At the same instance, 5ml of 4% boric acid (H_3BO_3, AR grade) was placed in the titration unit while adding the Kjeldahl indicator (Bromocresol Green and Methyl red in Methanol). Distillation was continued for 2 minutes and the liberated ammonia was trapped.

III. Titration

The resultant solution was titrated with 0.1M Hydrochloric acid (HCl, AR grade) until reaching the endpoint of colour transition from a green colour to purple. The same procedure was carried out for a blank sample as well.

The following equation was used to calculate the protein content of samples.

$$Nitrogen\ (\%) = \frac{(Sample\ titre - Blank\ titre)\ X\ Molarity\ HCl\ X\ 14}{Sample\ weight\ X\ 1000} X\ 100$$

The protein content is equal to the Nitrogen content X a pre-determined factor; 6.25. Results were then compared against the control sample.

Statistical analysis

Data collected through the above methodologies were statistically analyzed based on ANOVA at a 95% significance level, by using MINITAB 18 statistical software.

Results

As per the treatments of table 01 and table 02, from A1 to A11 samples, seed germination percentage is demonstrated in Figure-1.

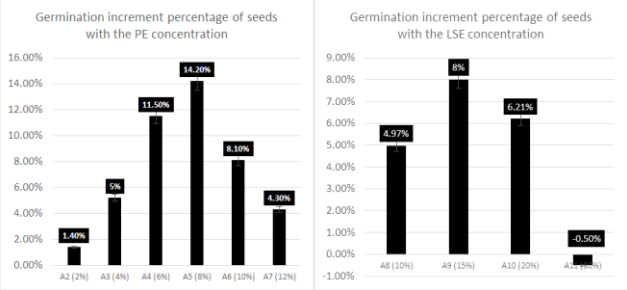

Figure-1. Increment of seed germination percentage against the control (A1), with PE and LSE, concentrates

Data stipulated in figure-1 depicts a significant difference at each PE and LSE concentration at $p<0.05$ level.

Germination was calculated during the stipulated 48-hour time period and as per the observations, the highest germination percentage was observed in the PE with 8% concentration, followed by the 15% concentration of LSE. At 30% LSE concentration germination level has dropped below that of the control. After observing the germination percentage, observations were made at the growth lengths of root & shoot.

The LSE treated samples have shown a comparative growth when comparing with the control. However, it is also observed that the concentration of the treatments has played a crucial role in seedling growth. Subjected to extreme concentrations, the percentage of germination was less due to the increased respiratory activities. But at the moderate concentration, respiratory activities are also considered to be moderate and the germination has considerably increased (Table-4). In both shoot and root, the highest increment of length is observed in the A9 sample, where the LSE is reached for 15%. In this sample, growth increment against the control is 8.7%, 8.3% & 12% respectively for shoot growth after 3, 4, and 5 days. In terms of the root growth, growth percentage is 9%, 7.1%, and 8.7% for 3, 4, and 5 days respectively. Similarly, the A5 sample denotes the PE concentration of 8% and has shown significant growth over the control. It shows a growth increment of 13% within the first 3 days against the control and 12.5% and 12% for the following 4^{th} and 5^{th} days respectively. When considering the root, the growth was 7%, 8.9%, and 8.7% for the consecutive days respectively. The highest values of root and shoot growth increment are significantly higher than the control and reduce with the further increment of the concentration.

For the foliar application given at the flowering stage, samples showed an obvious improvement of yield, against the control sample (Table-5).

Pods were collected at maturity from each plant and have shown an increment until the 10% LSE and PE concentration. The highest incremental number of pods was observed at 10% at both applications being 28% and 31% respectively. When the concentration was increased from this level, the yield has dropped significantly. Similarly, the number of seeds per pod has reached the maximum at the 10% level with 4.7% and 5.6% respectively for LSE and PE. Seeds were weighed after drying in the oven at 70°C, to obtain a constant weight and the seed weight has reached the maximum with an increment of 6.8% (LSE) and 7.6% (PE). However, seed weight increment has no significant difference against each treatment. Considering all the contributors, it is evident that the increment of the yield is mainly due to the increment of the seed yield and the number of pods per plant.

However, pod yield and the seed yield per pod along with the weight of the seed have increased with the application of LSE and PE and have reached its maximum at the 10% application of LSE and PE both.

Mineral and the protein content results have shown a significant difference with the application of the seaweed extract (Table-6). However, with the increment of the LSE and PE concentration, these significant differences were not observed. The highest mineral and protein content is observed in the 10% concentrated application of LSE and PE. Similar to the yield of pods and seeds when increasing the concentration of both PE and LSE, mineral and protein content decrease as well.

Table-4. Germination (root & shoot lengths) of samples from Al to All

Sample	Shoot Length (cm)						Root Length (cm)					
	3 Days		4 Days		5 Days		3 Days		4 Days		5 Days	
	Length	95% CI	Length	95% CI	Length	95% CI	Length	95% CI	Length	95% CI	Length	95% CI
A1	2.3 ± 0.1^{cd}	2.2132,2.3228	2.4 ± 0.0^{ab}	2.3242,2.4118	2.5 ± 0.1^{cd}	2.4148,2.5052	5.6 ± 0.1^{e}	5.5123,5.5917	5.6 ± 0.0^{e}	5.5113,5.5927	5.7 ± 0.0^{d}	5.6124,5.6996
A2	2.3 ± 0.1^{cd}	2.2132,2.3228	2.5 ± 0.0^{a}	2.4082,2.4958	2.5 ± 0.1^{cd}	2.4068,2.4972	5.8 ± 0.0^{c}	5.7763,5.8557	5.8 ± 0.1^{bc}	5.7753,5.8567	5.9 ± 0.1^{c}	5.8084,5.8956
A3	2.3 ± 0.2^{cd}	2.2252,2.3348	2.5 ± 0.1^{a}	2.4082,2.4958	2.6 ± 0.1^{bc}	2.5108,2.6012	5.9 ± 0.0^{bc}	5.8643,5.9437	5.9 ± 0.1^{b}	5.8633,5.9447	6.0 ± 0.0^{b}	5.9124,5.9996
A4	2.4 ± 0.2^{bc}	2.3292,2.4388	2.6 ± 0.2^{b}	2.5082,2.5958	2.7 ± 0.1^{ab}	2.6108,2.7012	6.0 ± 0.2^{a}	5.9803,6.0597	6.0 ± 0.0^{a}	5.9793,6.0607	6.1 ± 0.0^{ab}	6.0124,6.0996
A5	2.6 ± 0.1^{a}	2.5092,2.6188	2.7 ± 0.1^{a}	2.6122,2.6998	2.8 ± 0.1^{a}	2.7068,2.7972	6.0 ± 0.1^{a}	5.9843,6.0637	6.1 ± 0.0^{a}	6.0273,6.1087	6.2 ± 0.1^{a}	6.1084,6.1956
A6	2.1 ± 0.1^{e}	2.0172,2.1268	2.1 ± 0.0^{d}	2.0282,2.1158	2.2 ± 0.2^{e}	2.1068,2.1972	5.9 ± 0.1^{b}	5.8883,5.9677	5.8 ± 0.1^{bc}	5.8033,5.8847	5.8 ± 0.1^{c}	5.8004,5.8876
A7	2.0 ± 0.1^{e}	1.9372,2.0468	2.0 ± 0.1^{d}	1.9482,2.0358	2.0 ± 0.2^{f}	1.9468,2.0372	5.7 ± 0.0^{d}	5.6803,5.7597	5.8 ± 0.0^{c}	5.7113,5.7927	5.8 ± 0.0^{cd}	5.7084,5.7956
A8	2.3 ± 0.1^{d}	2.1972,2.3068	2.5 ± 0.2^{b}	2.4362,2.5238	2.5 ± 0.1^{c}	2.4348,2.5252	5.7 ± 0.0^{d}	5.6683,5.7477	5.6 ± 0.1^{de}	5.6033,5.6847	5.8 ± 0.0^{c}	5.7164,5.8036
A9	2.5 ± 0.1^{ab}	2.4532,2.5628	2.6 ± 0.0^{b}	2.5082,2.5958	2.8 ± 0.1^{a}	2.7068,2.7972	6.1 ± 0.2^{a}	6.0563,6.1357	6.0 ± 0.1^{a}	6.0073,6.0887	6.2 ± 0.1^{a}	6.1124,6.1996
A10	2.3 ± 0.0^{cd}	2.2052,2.3148	2.4 ±0.1 ^{ab}	2.3202,2.4078	2.5 ± 0.1^{cd}	2.4068,2.4972	5.5 ± 0.0^{e}	5.4723,5.5517	5.6 ± 0.2^{de}	5.5313,5.6127	5.8 ± 0.0^{c}	5.7204,5.8076
A11	2.1 ± 0.1^{e}	2.0132,2.1228	2.1 ± 0.1^{d}	2.0242,2.1118	2.4 ± 0.1^{d}	2.3108,2.4012	5.5 ± 0.1^{e}	5.5083,5.5877	5.7 ± 0.0^{d}	5.6113,5.6927	5.8 ± 0.1^{c}	5.7204,5.8076

Mean ± Standard deviation of 65 samples from root & shoot; significant difference among rows were denoted by different superscripts ($p<0.05$) on the length basis against A1 control. Means within the same row & column that have no common letters denote statistically significant differences among the figures concerned.

Table-5. Yield of pods and seeds after foliar application of LSE & PE

Sample	No. of pods per plant	95% CI	No. of seeds per pod	95% CI	Seed yield per plant (g)	95% CI	Weight of 100 seeds (g)	95% CI
A6	18.92 ± 0.7^{d}	18.542,19.308	8.51 ± 0.4 ^{bc}	8.327,8.709	12.51 ± 0.4^{e}	12.3247,12.6973	3.98 ± 0.2^{bcd}	3.8472,4.0928
A12	21.53 ± 0.4^{c}	21.134,21.900	8.64 ± 0.1 ^{ab}	8.437,8.819	15.94 ± 0.1^{b}	15.7597,16.1323	4.12 ± 0.4^{abc}	4.0012,4.2468
A13	24.21 ±0.3^{a}	23.840,24.606	8.91 ± 0.1^{ab}	8.7234,9.1046	16.13 ± 0.2^{b}	15.958,16.330	4.25 ± 0.4^{a}	4.1422,4.3878
A14	19.53 ± 0.1^{d}	19.125,19.891	8.73 ± 0.2 ^{ab}	8.543,8.925	15.87 ± 0.1^{b}	15.6807,16.0533	4.08 ± 0.2^{abc}	3.9582,4.2038
A15	17.54 ± 0.1^{e}	17.146,17.912	8.60 ± 0.1 ^{ab}	8.4184,8.7996	13.45 ± 0.2^{d}	13.273,13.645	3.65 ± 0.3^{e}	3.5232,3.7688
A16	19.17 ± 0.5^{d}	18.781,19.547	8.65 ± 0.1 ^{ab}	8.4494,8.8306	14.67 ± 0.4^{c}	14.4777,14.8503	3.74 ± 0.2^{de}	3.6192,3.8648
A17	20.96 ± 0.5^{c}	20.570,21.336	8.70 ± 0.2 ^{ab}	8.517,8.899	16.11 ± 0.7^{b}	15.9117,16.2843	3.95 ± 0.1^{cd}	3.8272,4.0728
A18	22.43 ± 0.4^{b}	22.036,22.802	8.71 ± 0.2 ^{ab}	8.5224,8.9036	16.67 ± 0.5^{a}	16.4707,16.8433	4.10 ± 0.0^{abc}	3.9802,4.2258
A19	24.76 ± 0.8^{a}	24.370,25.136	8.99 ± 0.1^{a}	8.7964,9.1776	16.84 ± 0.6^{a}	16.6427,17.0153	4.31 ± 0.2^{a}	4.1892,4.4348
A20	19.34 ± 0.4^{d}	18.971,19.737	8.10 ± 0.2^{c}	7.8944,8.2756	16.10 ± 0.5^{b}	15.9197,16.2923	4.25 ± 0.3^{ab}	4.1252,4.3708

Mean ± Standard deviation from 200 sample plants; significant difference among rows were denoted by different superscripts ($p<0.05$) against A6 control. Means within the same column that have no common letters denote statistically significant differences among the figures concerned against the control.

Table-6. Mineral and protein content of the seeds of LSE and PE treated samples

Mineral and protein content in mg per 100g of *Vigna radiata* seed flour												
Sample	**Ca**	**95% CI**	**Fe**	**95% CI**	**Mg**	**95% CI**	**K**	**95% CI**	**Na**	**95% CI**	**Protein**	**95% CI**
A6	78.1 ± 0.0^{d}	77.376,78.764	8.1 ± 0.0^{e}	7.9817,8.2183	50.1 ± 0.0^{i}	49.477,50.643	335.4 ± 0.0^{e}	334.562,336.338	10.3 ± 0.0^{f}	10.2548,10.4252	21.0 ± 0.1^{e}	20.525,21.455
A12	79.3 ± 0.1^{bcd}	78.566,79.954	8.4 ± 0.0^{d}	8.3217,8.5583	50.8 ± 0.0^{efg}	50.247,51.413	338.7 ± 0.0^{cd}	337.832,339.608	11.6 ± 0.0^{d}	11.5048,11.6752	20.7 ± 0.2^{e}	20.245,21.175
A13	80.2 ± 0.0^{b}	79.486,80.874	9.0 ± 0.1^{bc}	8.8307,9.0673	53.8 ± 0.1^{bc}	53.187,54.353	341.2 ± 0.0^{b}	340.862,342.638	12.4 ± 0.1^{a}	12.3448,12.5152	24.2 ± 0.1^{b}	23.765,24.695
A14	78.5 ± 0.0^{cd}	77.826,79.214	8.8 ± 0.1^{bc}	8.6937,8.9303	51.6 ± 0.1^{ef}	50.997,52.163	338.7 ± 0.0^{cd}	337.852,339.628	11.8 ± 0.1^{c}	11.734811.9052	21.8 ± 0.3^{de}	21.295,22.225
A15	79.2 ± 0.1^{bcd}	78.486,79.874	8.8 ± 0.0^{bc}	8.7117,8.9483	50.4 ± 0.0^{fg}	49.827,50.993	337.2 ± 0.1^{de}	336.362,338.138	10.4 ± 0.1^{f}	10.3148,10.4852	21.0 ± 0.2^{e}	20.575,21.505
A16	80.1 ± 0.1^{bc}	79.426,80.814	8.7 ± 0.0^{c}	8.6117,8.8483	50.2 ± 0.0^{g}	49.577,50.743	340.8 ± 0.1^{bc}	339.872,341.648	11.0 ± 0.0^{e}	10.9448,11.1152	22.3 ± 0.1^{d}	21.795,22.725
A17	80.1 ± 0.0^{b}	79.436,80.824	8.9 ± 0.0^{bc}	8.7917,9.0283	52.1 ± 0.1^{de}	51.517,52.683	340.7 ± 0.1^{bc}	339.782,341.558	11.2 ± 0.0^{e}	11.1248,11.2952	22.5 ± 0.2^{cd}	21.985,22.915
A18	82.4 ± 0.0^{a}	81.666,83.054	9.1 ± 0.1^{b}	8.9417,9.1783	54.9 ± 0.1^{ab}	54.357,55.523	342.5 ± 0.0^{b}	341.662,343.438	12.1 ± 0.0^{b}	11.9748,12.1452	23.4 ± 0.2^{bc}	22.935,23.865
A19	83.7 ± 0.1^{a}	83.006,84.394	9.4 ± 0.1^{a}	9.2517,9.4883	55.6 ± 0.0^{a}	54.977,56.143	345.8 ± 0.0^{a}	344.912,346.688	12.4 ± 0.1^{a}	12.3248,12.4952	25.4 ± 0.3^{a}	24.955,25.885
A20	80.0 ± 0.0^{bc}	79.342,80.730	9.1 ± 0.1^{b}	8.9317,9.1683	53.0 ± 0.0^{cd}	52.437,53.603	338.6 ± 0.0^{d}	337.672,339.448	11.8 ± 0.1^{c}	11.7448,11.9152	24.1 ± 0.1^{b}	23.675,24.605

Mean ± Standard deviation from Vigna radiata seeds; significant difference among columns were denoted by different superscripts ($p<0.05$) against A6 control. Means within the same column that have no common letters denote statistically significant differences among the figures concerned against the control.

Discussion

Polysaccharide and crude seaweed extractions of *Sargassum* sp. are used in this study to identify their impact on the rate of germination, growth, pod and seed yield, and nutrient content analysis of *Vigna Radiata* seeds. Increasing the yield in crops, with the application of seaweed extracts are reported for *Capsicum annum* (Arthur et al., 2003), *Zea mays* (Rajkumar and Subramanian, 1999) & *Brassica napus* (Ferreira and Lourens, 2002). In a parallel study, the seaweed *Kappaphycus* has increased the yield of all concentrations of nutrient supply by increasing the weight of the bean (Beckett et al., 1994). The increment in the germination, growth, and yield in low concentration of seaweed extracts might occur due to the presence of gibberellin, cytokinin like growth regulators, phenylacetic acid (Sivasankari et al., 2006), and micronutrients (Layek et al., 2014).

Soaking the seeds with lower concentrations of the extracts showed an improvement of growth and yield factors against the control. The highest germination percentage was observed in PE with 8% concentration and in LSE with a 15% concentration. This signifies that a PE with lesser concentration can achieve a stipulated growth when comparing with LSE. Therefore, quantity-wise, it will be more beneficial to use PE than LSE, in crop production. However, at 20% concentration of LSE and 10% concentration of PE, the germination rate has been initiated to reduce, compared with the control. This signifies that there is an optimum concentration of LSE and PE, which could be used in crop production. It is evident that unless used at the optimum concentration, higher dosages of both PE and LSE will reduce the rate of germination due to the excessive stress resulting reduction of growth rate (Sivasankari et al., 2006).

When considering the growth attributes including the root and shoot length, maximum growth was observed at 8% polysaccharide and 15% crude seaweed extract concentration. At LSE 15% concentration, the root had an average growth of 8.3%, while the shoot had an average growth of 9.6% against the control. At 8% PE, the shoot had a 12.5% growth while the root had 8.2% average growth against the control. This observation in obtaining the highest growth at comparatively a low concentration of PE, compared to LSE is due to the concentrated amount of growth regulators in PE against LSE, which might have advanced the photosynthesis thereby stimulating vegetative growth (Devi and Mani, 2015). This observation has also been made for gram (Pramanick et al., 2013) and wheat (Shah et al., 2013). Further to these studies, it is also highlighted that the presence of bioactive substances is likely to improve the intake of stomatal uptake efficiency (Mancuso et al., 2006; Rathore et al., 2009). Since both PE and LSE had concentrated levels of bioactive substances, it is likely to have impacted the plant growth positively. Also, the presence of growth regulators advances photosynthesis, thereby increasing the growth of crops. However, overdosing of the LSE and PE has impacted the growth rate

similar to the germination rate, stressing the fact that excessive stress can demote the crop growth rate.

When considering the improvement of the seed and pod yield, 10% was the LSE and PE extraction concentration which gave the highest yield against the control. Yield has improved up to 31% from these applications, which is statistically significantly different from one another as well. Weights of the grains have also increased significantly and have reached the highest at 10% concentration at both of the application types. The result signifies that the yield of the *Vigna radiata* crop has increased significantly when applying LSE and PE. The number of pods per plant as well as the number of seeds per pod has increased significantly, thereby increasing the number of seeds per plant.

These observations could be related to the movement of the resultants of photosynthesis from the vegetative parts to the development parts of the plant; grains (Shah et al., 2013). The observation could be justified by another study done on the green gram (Pramanick et al., 2013) by the application of *Kappaphycus alvarezii* and *Gracilaria edulis*.

Protein content analysis of seeds has resulted in obtaining the highest yield from 10% application of both LSE and PE. Also, mineral contents of Ca, Fe, Mg, K, Na has increased with the increasing levels of the application concentration and have reached the maximum at 10% concentration. This may be due to the ability of *Sargassum* to increase nutrient utilization from the soil while adding as an additional mineral source to the crop.

Similar results have been observed in *Vitis vinifera* and *Cucumis sativus* with the seaweed applications (Turan and Kose, 2004; Mancuso et al., 2006). The reason for the high mineral and nutrient content after seaweed application is highlighted as due to the ability of seaweeds to enhance the effectiveness of fertilizers by increasing nutrient utilization by soil (Frankenberger and Arshad, 1995; Sharma et al., 2014). When considering the protein content, the increment is around 20% for both LSE and PE and is significantly different from the control. The same result has been reported for *Vigna catajung* (Anantharaj and Venkatesalu, 2001) and the result is justified by the root proliferation and thereby an increment in N, P, and S uptake, which is needed for protein synthesis. It was also observed that the seaweed applications have increased micronutrient content in rice grains like Cu and Zn up to 10% concentrations and Fe and Mn up to 5% concentrations (Layek et al., 2014).

Conclusion

With these analytical results, it is evident that the extractions of seaweeds could be used to improve the rate of seed germination, growth, and the yield of plants. Based on the result of this study, it is recommended to use the LSE at 15% concentration and polysaccharide extraction at 8% concentration at the germination stage and as a foliar application to increase the yield and nutrient content of the *Vigna radiata* plant. Also, *Sargassum* sp. applications improve the mineral and protein uptake of plants and thereby impacting the mineral and protein levels of the *Vigna radiata* seed crop.

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Contribution of Authors

Makawita GIPS: Conceived idea, designed researched methodology, data collection and analysis and manuscript writing

Wickramasinghe I & Wijesekara I: Helped in designing the experiment, data analysis, data interpretation and manuscript writing