Recovery ratio and quality of an agricultural bio-stimulant and semi-refined carrageenan co-produced from the fresh biomass of *Kappaphycus alvarezii* with respect to seasonality

M. Shanmugam*, Abhiram Seth

Research and Development Division (DSIR Lab), AquAgri Processing Private Limited, #B5, SIPCOT Industrial Complex, Manamadurai - 630 606, Sivaganga District, Tamil Nadu, India

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**ABSTRACT**

In general, to date, the majority of cultivated, dried biomass of *Kappaphycus* spp. has been used for the manufacture of refined (RC) or semi-refined carrageenan (SRC) on a commercial scale. However, the present study was focused on the production of SRC using wet fibrous residue left over after an agricultural bio-stimulant was extracted from the fresh biomass of *Kappaphycus alvarezii* in the manner of a biorefinery operation. The investigation examined the recovery ratio and quality of both bio-stimulant and SRC from the fresh biomass of *K. alvarezii* farmed on the Tamil coast of India for two consecutive seasons. This study was conducted on a large, pre-commercialization pilot-scale, i.e. 10.0 t of fresh biomass of *K. alvarezii* was crushed in each batch and a total of 10 batches were made, each month during 2012 and 2013. The total recovery of the agricultural bio-stimulant and SRC from 1.0 t of fresh material processed at different seasons ranged from 19.58–23.69 dry kg (average 21.09 ± 1.35 kg) and 24.27–41.61 dry kg (average 31.75 ± 6.99 kg) respectively with a moisture content of 934.70–956.15 kg (average 947 ± 6.17 kg). The economics of conversion of fresh seaweed into agricultural bio-stimulant and SRC through MUZE (multi-stream, zero-effluent) process was far better as compared to conversion of dry weed into SRC alone through conventional methods, i.e. 1.0 t FS (Fresh seaweed) yielded a net profit of 224.14 US$ through multi-stream processing, whereas its dry-weed equivalent produced only 118.34 US$. The present investigation concluded that the fresh biomass of *K. alvarezii* can be used to co-produce agricultural bio-stimulants with a good efficacy and provide a relatively low yield of SRC with medium gel strength.

1. Introduction

Bio-stimulants are substances, including microorganisms, which are applied to plants, seeds, soils or other growing media in order to enhance the ability of a plant to assimilate applied nutrients, or provide benefits to plant development and productivity [1]. According to Van Oosten et al., the bioeffector or bio-stimulant is an organic material and/or micro-organism that is applied to enhance nutrient uptake, stimulate growth, enhance stress tolerance or crop quality [2]. Bio-stimulants fall into five major categories, i.e. microbial inoculants, humic acids, fulvic acids, protein hydrolysates and amino acids, and seaweed extracts [2]. The demand for agricultural bio-stimulants is increasing annually due to their reported properties such as activating plant physiology, stimulating soil microbial function, and amending nutrients and pH in the rhizosphere. Their market potential is projected to reach USD 2.91 Billion by 2021, with a CAGR of 10.4% from 2016 to 2021 [3]. Seaweed biomass is receiving increasing attention from biotechnology developers in global technology centers for their value-added products. Production of semi-refined carrageenan using conventional technology from raw dried seaweed (RDS) is common practice in a number of areas of the world. However, more recently, Neish introduced the concept of multi-stream, zero-effluent (MUZE) technology that commences processing using live, fresh seaweed (FS) in order to produce not only SRC but also agricultural inputs that may be possible from developing technology [4].

The use of bio-stimulants for agricultural purposes is becoming widespread and seaweed products have a special niche in this category. Seaweeds have been used for centuries, either directly or as a composted mixture, as a soil amendment in order to enhance soil fertility and crop productivity [5,6]. Following the initial development of a process to produce liquid extracts of seaweed in the 1950s [7], a variety of commercial seaweed extract products are now available worldwide for use in agriculture and horticulture [1,8].

Many of the bio-stimulant extracts currently available on the market are manufactured from brown seaweed resources such as *Ascophyllum*...
nodosum [9], Ecklonia, Macrocystis, Fucus, Laminaria, Sargassum and Turbinaria spp. [10]. But during the last decade, several authors have studied the bio-stimulatory effect of extracts from the red alga Kappaphycus alvarezii and demonstrated an increased yield and quality of a wide range of crops [11–19]. The bio-stimulant sap from K. alvarezii is rich in potash, along with other macro and micro nutrients and some suggested plant growth substances, viz. auxins, cytokinins and gibberellic acids [14,20].

The varied manufacturing processes for the production of commercial seaweed extracts include the use of water, acid, or alkali as extractants, with or without heating, or the physical disruption of seaweed tissues using low temperature milling or high pressure, cell-burst techniques [6,21]. The final product is prepared as a liquid or a dried powder and may be formulated with plant fertilizers (N, P and K) and micronutrients [6]. The chemical constituents of any seaweed extract include complex polysaccharides, oligomers, fatty acids, vitamins, phytohormone-like compounds and mineral nutrients [22].

Kappaphycus is extensively used for the industrial production of κ-carrageenan [23] and recently, semi-refined carrageenan (SRC) has been used as an alternative source of carrageenan which has been mostly applied in pet food and dairy industries where the clarity of the product is not an important issue. Furthermore, the product is also processed as per food safety procedures; therefore, SRC is permitted for human consumption [24,25]. About 0.05 Mt. of carrageenan is produced annually from over 0.2 Mt. of dry K. alvarezii and its market demand is growing at approximately 10% CAGR [26].

It is known that the chemical structure and biological activity of seaweed extracts vary according to the raw material, the extraction process, and the harvest season of algal material and many researchers have showed seasonal changes in the content of plant growth regulators in the extracts of various seaweeds [27–30]. Seasonal variation in the yield and quality of SRC from dried K. alvarezii has been recorded in the literature [31–33]. However, to date, there has been no study undertaken of SRC variations as manufactured from the fresh biomass of K. alvarezii.

The present study examined variations in the recovery ratio and quality of an agricultural bio-stimulant sap and semi-refined carrageenan as co-produced from fresh biomass of K. alvarezii, as farmed in Tamil Nadu, India and also provided an analysis of the mass balance with respect to seasonality for the first time.

2. Materials and methods

The commercial farming of K. alvarezii has been conducted in Tamil Nadu since 2001 [26] and AquaAgri Processing (P) Ltd., a seaweed-based industry in India has been co-producing SRC and bio-stimulant sap from fresh biomass by adopting a patented technology [34]. Fresh K. alvarezii (45–50 d under cultivation) was procured monthly from 2012 to 2013 from Mandapam (9.28°; N 79.12°E) and used for the co-production of bio-stimulant sap and SRC. The prevailing seasons in Tamil Nadu are winter (Jan–Mar), summer (Apr–Jun), pre-monsoon (Jul–Sep) and monsoon (Oct–Dec). The harvested, fresh biomass was transported in a closed truck and generally it took 2–3 h from harvested point to processing unit.

2.1. Pulverization of the fresh biomass and separation of the bio-stimulant sap from the wet fibrous residue

10.0 ± 0.01 t of fresh K. alvarezii was quickly washed with fresh water to remove dirt and sand particles and was homogenized in a pulveriser (Fruit Juicer, Kalinga Engineer Ltd.; Capacity 2 hr−1, 600 mm diameter hopper, fitted with 40HP motor, Osissa, India) into a slurry, which was then passed through a D-canter (Pennwalt P3400 Model Super D-canter Centrifuge, Pennwalt Ltd. Mumbai, India) to separate the liquid from a wet fibrous residue. The liquid portion was then double filtered (Sand filter followed by ceramic filter) 0.02 mm pore; A.T.E. Envirotech (P) Ltd. Mumbai, India) to obtain a bio-stimulant sap and the solid residue obtained was returned to the wet fibrous residue for the production of SRC. The total recovery of the bio-stimulant (dry kg) from 1.0 t of fresh biomass was calculated based on the total volume of the bio-stimulant liquid obtained and its total soluble solid content (TSS). The wet fibrous residue (granule size ≤ 1.0 mm) was used for the production of SRC and its recovery per 1.0 t of FS was calculated. A total of 10 batches were run every month from January 2012 until December 2013 and seasonal variations in the yield and quality of both bio-stimulant sap and SRC were calculated.

2.2. Production and analysis of SRC from wet fibrous residue and whole dry-weed

Production of SRC using the wet fibrous residue as a raw material is described in the schematic diagram as shown in Fig. 1. The wet fibrous residue was cooked in 8.0% aqueous KOH solution (1:2 ratio) in a jacketed stainless steel tank (SS-316, 7KL capacity) at 70 °C ± 2 °C for 90 min. With mild agitation of 24 rpm [34,35]. The cooked material was separated using a vibrating screen (1500 mm dia, 200 mm height with 20 mesh screens, 20 ASTM 850 μm) and washed with water to remove any excess KOH; it was dried in a tunnel drier at 60 ± 2 °C for 8.0 h (Reny Marketing, Coimbatore, India) followed by vibratory fluidized bed drier at 80 ± 2 °C for 2.0 h (Teak Craft, Capacity 200 kg hr−1 with 36 HP motor, Coimbatore, India). The dried SRC granular material was ground in a microniser (Septu India (P) Ltd. Haryana, India) and sieved through an 80 mesh (80 A.S.T.M, 180 μm) in order to obtain SRC samples for further testing [Shanmugam Personal communication]. The size of an individual SRC batch was 2000 ± 20 kg and a total 30 batches were run during each season (10 batches per month) and their average values presented. SRC from whole, dry-weed of K. alvarezii was manufactured as per the method described by Shanmugam et al. [35] for the comparison of data with SRC prepared from wet fibrous residue.

The raw material was stuffed into perforated basket and pre-washed with water for 30 min and cooked with 9% KOH at 80 ± 2 °C for 2 h. The cooked material was then washed with water to remove excess KOH, chopped into 2–5 mm particles and sun-dried followed by fluidized bed drier. The dried SRC chips were ground and sieved through an 80 mesh sieve (180 A.S.T.M) to obtain SRC samples for further testing. A total of 10 batches (batch size of 1000 ± 10 kg) were run in the present investigation and their average values reported.

A moisture-free SRC sample was prepared at 85 ± 2 °C for 16.0 h and incinerated in a muffle furnace at 550 °C for 4 h; the ash content was determined gravimetrically [36]. The ester sulfate content was estimated by hydrolyzing the sample followed by precipitation of sulfate as BaSO4 [36] and calculated using the following equation:

\[
%\text{sulfate} = \left(\frac{W_2}{W_1}\right) \times 100 \times 0.4116,
\]

where \(W_1\) = initial weight of the SRC sample and \(W_2\) = weight of ash as BaSO4.

The acid insoluble matter of SRC was determined by treating the sample with 0.1% sulfuric acid as described by Mehta et al. [25]. Viscosity was measured at 1.5% in distilled water at 75 °C, 30 rpm and spindle no.62 using a Brookfield LVDV-II + pro. KCI modified gel strength (KGS) was determined at 1.5% of SRC in 0.2% KCl solution using a Brookfield Texture Analyzer (Model CT3 4500) and the native water gel strength (WGS) was measured in same way using distilled water without KCl [31,35]. The 3.6-anhydrogalactose content of the SRC samples was estimated by an improved, phenol-resorcinol method using fructose as the standard [37]. The microbial load of the SRC samples was measured by inoculating 0.1 ml of 1% SRC solution into nutrient medium (i.e. SS agar for Salmonella and Shigella, EMB for E. coli and SDA for yeast and mould) incubated for 48 h and the colony forming units (CFU) per gram of SRC were counted [38]. The FT-IR spectra of the SRC samples were analyzed in KBr pellets using a Perkin-Elmer Spectrophotometer GX.
2.3. Analysis of bio-stimulant sap and studies of its efficacy on the germination of maize seeds

The total soluble solid content (TSS) of the bio-stimulant sap was measured by a gravimetric method [14], i.e. a known volume of bio-stimulant sap was dried in oven at 80 ± 2 °C for 4 h or till constant weight was maintained and determined its TSS % and its pH measured using a digital pH meter (Model: Eutech Instrument). An elemental analysis was performed by flame photometer and atomic absorption spectroscopy. The plant growth regulators (PGRs) such as auxins, gibberellins, and cytokinins were extracted from the bio-stimulant sap using diethyl ether, ethyl acetate, and n-butanol respectively and standard samples were prepared as follow: 4.4 mg of IAA in 2 ml of MeOH, 1 mg of kinetin in 10 ml of MeOH/H₂O (9:1 v/v), 1 mg of trans-

![Production flow chart of SRC from wet fibrous residue obtained after extraction of bio-stimulant sap from K. alvarezi.](image1)

![Effect of bio-stimulant sap manufactured from the fresh biomass of K. alvarezi on the germination of maize seeds.](image2)
zeatin in 10 ml of MeOH/H2O (9:1 v/v), and 1.7 mg of GA3 in 2 ml of MeOH. Standards were put on to TCL plates with corresponding extracts of bio-stimulant sap and quantification assay was done using HPLC technique as described by Prasad et al. [20].

Experiments were conducted using certified maize seeds (Zea mays) obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, India. Experimental units were arranged in a randomized, complete block design and four groups of 150 certified seeds were tested for germination studies per each treatment (Fig. 2). The seeds were soaked in different concentrations of bio-stimulant sap, i.e. 0.3, 0.5% and 0.7% for 1 h and placed in a plastic cup (100 g capacity) filled with sterile coco-peat and germinated in a chamber providing 12 h photoperiod and a diurnal temperature range of 32/27 °C with the seeds being watered every 12 h. Germination was considered to have occurred once the radicle protruded > 2 mm. Germination was observed daily over 6 days and variables such as percentage germination \( [PG = (\text{number of germinated seeds}/\text{number of total seeds per treatment after 48 h} \times 100] \), germination energy \( [\text{GE} \% = (\text{number of germinated seeds}/\text{number of total seeds per treatment after 48 h} \times 100] \) and seedling vigor index \([\text{SVI} = (\text{seedling length (mm)} \times \text{germination percentage}] [39] \).

2.4. Statistical analysis

Data are presented as means ± SD of at least nine independent measurements. A one-way analysis of variance (ANOVA, SYSTAT version 7) was used to determine the effect of season on the recovery of SRC and the bio-stimulant sap and quality parameters. A Tukey’s HSD test was applied for post-hoc comparison studies and data were considered statistically significant when \( P < 0.05 \).
3. Results

3.1. The recovery ratio and mass balance with respect to seasonality

The average volume of bio-stimulant sap obtained from the winter, summer, pre-monsoon and monsoon samples corresponded to: 575–585, 438–495, 465–510 and 576–605 kg 1.0 t−1 FS with an average total soluble solid content (TSS %) content of 3.94–4.05, 4.47–4.15, 4.23–4.11 and 3.52–3.53% respectively (Figs. 3a,b & 4a,b). Therefore, the total dry bio-stimulant recoverable (kg) from 1.0 t FS on a seasonal basis were within the range of 22.66–23.69 kg (winter), 19.58–20.54 kg (summer), 19.67–20.96 kg (pre-monsoon) and 20.28–21.36 kg (monsoon) i.e. the winter-monsoon samples yielded 20.97% more bio-stimulant sap than the summer-pre-monsoon samples. The average wet fibrous residue obtained was 360–375, 450–485, 445–463 kg 1.0 t−1 FS in winter, summer, pre-monsoon and monsoon months respectively. There was a SRC recovery of 24.94–26.60 (winter), 37.80–41.61 (summer), 36.27–38.61 (pre-monsoon) and 24.27–24.30 kg (monsoon) 1.0 t−1 FS. Thus, the overall yield of SRC ranged between 6.65 and 8.58%. The recovery of bio-stimulant was significantly (F = 64.22; p = 0.0004) higher in samples from the winter and monsoon months, than those from the summer and pre-monsoon months and the reverse trend was evident in the recovery of SRC, i.e. higher recovery of SRC from samples taken during the summer and pre-monsoon periods, than other two seasons (F = 196.09; p = 0.0001). Therefore, the recovery of bio-stimulant and SRC together from fresh K. alvarezii (under 45–50 d cultivation) harvested from different season was only 45–60 kg from 1.0 t of fresh biomass with the remaining 940–955 kg being moisture (Table 1).

3.2. Quality of the bio-stimulant sap and its efficacy

The physico-chemical properties and PGR content of the bio-stimulant sap are given Table 2. The TSS % of the bio-stimulant manufactured from summer and pre-monsoon samples were nominally higher than those from the winter and monsoon samples. However, these were not statistically significant. The pH of the liquids produced over the different seasons ranged between 6.50 ± 0.05–7.20 ± 0.10 without significant differences across those seasons. Similarly, no variation in the specific gravity of the liquid was determined; it was 1.03 ± 0.002. The total organic matter content of the bio-stimulant sap produced in the winter, summer, pre-monsoon and the monsoon months of 2012 and 2013 ranged between 0.65 ± 0.04 to 0.81 ± 0.05%, i.e. no significant variation in the content for entire study period of two years. The total ash content was marginally higher in the summer and pre-monsoon samples (3.63–3.70%) than the other two seasons (2.72–3.4%) and it was positively correlated to the TSS content of the bio-stimulant sap (p = 0.003).

Amongst the primary nutrients, potassium was a major constituent of all the samples tested, it was marginally higher in the summer - pre-monsoon seasons (1.62 ± 0.05–1.70 ± 0.10%) than those from the winter - monsoon period (1.46 ± 0.11–1.55 ± 0.08%). This was positively correlated to the TSS of the bio-stimulant sap and the same trend was observed with the nitrogen (180 ± 22–298 ± 3 ppm) and phosphorous contents (14 ± 2–38 ± 3 ppm) during the four seasons, over the two years studied. Calcium levels were reported as:

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Bio-stimulant liquid per 1.0 t of fresh biomass (kg)</th>
<th>Total solid content of bio-stimulant (%)</th>
<th>Total bio-stimulant recoverable per 1.0 t of fresh biomass (dry kg)</th>
<th>Semi-refined carrageenan (SRC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wet fibrous residue per 1.0 t of fresh biomass (kg)</td>
</tr>
<tr>
<td>2012 Winter</td>
<td>575 ± 34.5</td>
<td>3.94 ± 0.23</td>
<td>22.66 ± 1.83a</td>
<td>360 ± 24.48</td>
</tr>
<tr>
<td>Summer</td>
<td>438 ± 21.9</td>
<td>4.47 ± 0.26</td>
<td>19.58 ± 2.11a</td>
<td>485 ± 27.34</td>
</tr>
<tr>
<td>Pre-monsoon</td>
<td>465 ± 28.3</td>
<td>4.23 ± 0.19</td>
<td>19.67 ± 2.70</td>
<td>463 ± 26.40</td>
</tr>
<tr>
<td>Monsoon</td>
<td>576 ± 38.0</td>
<td>3.52 ± 0.20</td>
<td>20.28 ± 2.55ab</td>
<td>365 ± 29.05</td>
</tr>
<tr>
<td>2013 Winter</td>
<td>585 ± 41.5</td>
<td>4.05 ± 0.21</td>
<td>23.69 ± 2.15a</td>
<td>375 ± 20.26</td>
</tr>
<tr>
<td>Summer</td>
<td>495 ± 28.1</td>
<td>4.15 ± 0.32</td>
<td>20.54 ± 1.72</td>
<td>450 ± 27.45</td>
</tr>
<tr>
<td>Pre-monsoon</td>
<td>510 ± 39.7</td>
<td>4.11 ± 0.25</td>
<td>20.96 ± 1.68</td>
<td>445 ± 28.84</td>
</tr>
<tr>
<td>Monsoon</td>
<td>605 ± 47.7</td>
<td>3.53 ± 0.27</td>
<td>21.36 ± 1.74ab</td>
<td>360 ± 31.80</td>
</tr>
</tbody>
</table>

Means followed by same letter (or no letter) are not significant at the 0.05 probability level.

Fig. 4. a. Seasonal variation in recovery of bio-stimulant sap and SRC (semi-refined carrageenan) obtained from 1.0 t fresh biomass of K. alvarezii farmed on the Tamil Nadu coast of India during different seasons of 2012. b. Seasonal variation in recovery of SRC (semi-refined carrageenan) and bio-stimulant sap obtained from 1.0 t fresh biomass of K. alvarezii farmed on the Tamil Nadu coast of India during different seasons of 2013.
Physico-chemical and PGR (Plant Growth Regulators) content of bio-stimulant manufactured from fresh K. alvarezii farmed in different seasons of 2012 and 2013 (SD ± ; n = 9).

<table>
<thead>
<tr>
<th>Physico-chemical parameters and PGRs</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>Total organic matter (g 100 g⁻¹)</td>
<td>0.70 ± 0.04</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>pH (1% solution)</td>
<td>6.81 ± 0.02</td>
<td>6.78 ± 0.08</td>
</tr>
<tr>
<td>Total soluble solid (g 100 g⁻¹)</td>
<td>3.94 ± 0.23</td>
<td>4.47 ± 0.26a</td>
</tr>
<tr>
<td>Total ash (g 100 g⁻¹)</td>
<td>3.24 ± 0.19</td>
<td>3.70 ± 0.15</td>
</tr>
<tr>
<td>Specific gravity (g cm⁻³)</td>
<td>1.03 ± 0.02</td>
<td>1.03 ± 0.003</td>
</tr>
<tr>
<td>Primary nutrients</td>
<td>Nitrogen (N) (g 100 g⁻¹)</td>
<td>185 ± 45</td>
</tr>
<tr>
<td>Phosphorous (P) (g 100 g⁻¹)</td>
<td>14 ± 2.0</td>
<td>27 ± 4.50</td>
</tr>
<tr>
<td>Potassium (K) (g 100 g⁻¹)</td>
<td>1.46 ± 0.11</td>
<td>1.69 ± 0.08a</td>
</tr>
<tr>
<td>Secondary nutrients</td>
<td>Calcium (Ca) (mg kg⁻¹)</td>
<td>266 ± 45</td>
</tr>
<tr>
<td>Magnesium (Mg) (mg kg⁻¹)</td>
<td>340 ± 65</td>
<td>330 ± 18</td>
</tr>
<tr>
<td>Sulphur (S) (mg kg⁻¹)</td>
<td>212 ± 20</td>
<td>265 ± 23</td>
</tr>
<tr>
<td>Plant growth regulators (PGRs)</td>
<td>Auxins (mg kg⁻¹)</td>
<td>55.05 ± 13.008</td>
</tr>
<tr>
<td>Cytokinins (mg kg⁻¹)</td>
<td>28.99 ± 15.90b</td>
<td>22.03 ± 11.50b</td>
</tr>
<tr>
<td>Gibberrellins (mg kg⁻¹)</td>
<td>123.8 ± 16.45</td>
<td>105.0 ± 12.50</td>
</tr>
</tbody>
</table>

Means followed by same letter (or no letter) are not significant at the 0.05 probability level.

The inter-annual variations of the yield and quality of both products were statistically insignificant (p = 0.18), therefore, the values of 2012 and 2013 were pooled for discussion. Bio-stimulant, SRC and the moisture content of the fresh K. alvarezii were distributed in a 2.16:3.28:94.56 ratio and recovery of both SRC and the bio-stimulant (dry kg) together per ton of fresh biomass was 59.79, 57.75, 48.96 and 45.14 kg with a moisture content of 940.14, 842.25, 951.04 and 954.86 kg from the summer, pre-monsoon, winter and monsoon months respectively. Therefore, 54.15% more SRC was obtained from the

4. Discussion
Bio-stimulants derived from seaweed raw materials have been used in agriculture as an innovative solution to address the challenges of sustainable agriculture, in order to assist nutrient uptake and improve crop yield, quality and provide a level of tolerance to abiotic stresses. However, it is important to understand the seasonal differences in the content and properties of such active compounds. It is known that the chemical structure and biological activity of seaweed extracts vary according to the harvest season of algal material [40,41]. In the present investigation, it was observed that the PGRs tested were found to be significantly higher in the winter-monsoon period which corresponded to the most active growth period of *K. alvarezii* and it is in agreement with previous reports [27,42-45]. Featonby-Smith and Van Staden [27] determined the seasonal variation of cytokinin content in the stipes of *Ecklonia maxima* in South Africa and found the highest values were recorded in winter (Apr - Jun) and summer (Nov - Dec) and that the lowest were found in the late summer to autumn period (Jan - Mar). Polyamines (PAs) are known to increase within terrestrial plant tissue mainly due to stress [42,43] and during the active growth period [44,45]. Papenfus et al. [29] reported that the level of PAs within *E. maxima* increased during the winter when stress occurred due to water temperature. Blunden et al., however, reported that there was no clear indication of any seasonal variation in the betaine yields of *Ascosiphillum nodosum* [28] and Stirk and Staden observed that cytokinin-like compounds decreased during the period of active growth for *E. maxima* [21].

The germination results showed no significant variation amongst the samples of the four seasons studied (Table 3). Several studies have examined the applications of seaweed extracts on seed germination of various species such as tomato [46], green gram [47] and wheat [30]. Increased germination percentage at low concentrations of seaweed extract could be due to the presence of growth-promoting substances such as indole acetic acid, indole-3-butyric acid, gibberellins, cytokinins, micronutrients, vitamins and amino acids [30]. The present results such as indole acetic acid, indole-3-butyric acid, gibberellins, cytokinins, micronutrients, vitamins and amino acids [30]. The present results are similar to those obtained by Mercer et al. [48] where maize grown at 10 °C germinated significantly faster when it had been pre-treated with a range of commercial seaweed extracts, as compared with water and dry controls. Karthikeyan and Shanmugam [14,15] reported that seedlings of banana and sugar cane pre-treated with extract of fresh *K. alvarezii* significantly improved the crop yield and quality. In general, only RDS are used for producing SRC by adapting the conventional method which requires the cooking of pre-washed, dried seaweed biomass in an aqueous, alkaline solution in a perforated basket. In the present investigation, the wet fibrous residue which remains after extraction of a bio-stimulant sap was used as the starting raw material for production of SRC. The method employed was different from the conventional system, i.e. pre-wash, cooking and post washes which were made in a closed circulation system (Fig. 1). In conventional extraction systems, the yield of SRC is reported to vary depending on the extraction method, the morphotype of the species and age of the raw material, thus quantitative and qualitative comparisons of methods and the efficiency of carrageenan production are difficult [31,33]. In the present study, with a wet fibrous residue being used as the starting raw material for extraction, the yield of SRC varied from 6.65 ± 0.48–8.58 ± 0.56% over the entire study period of two years. However, the yields of SRC, from dry *K. alvarezii*, using conventional methods have been reported as: 32.2–39.0% in Sri Lanka [38], 29.89–32.55% in India [36], 17.1–56.31% [49] and 45% [31] in Indonesia, 31–43% [50] and 41.16% [51] in Brazil, 34.5–45.30% in Vietnam, 54.5% in the Philippines [31] and 30.3–40.7% in Mexico [52].

### Table 3

<table>
<thead>
<tr>
<th>Season</th>
<th>Winter</th>
<th>Summer</th>
<th>Pre-monsoon</th>
<th>Monsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination percentage (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>56.57 ± 4.89</td>
<td>59.42 ± 3.69</td>
<td>57.17 ± 4.04</td>
<td>55.48 ± 3.71</td>
</tr>
<tr>
<td>0.5%</td>
<td>68.27 ± 7.24</td>
<td>69.05 ± 7.40</td>
<td>67.64 ± 7.22</td>
<td>65.12 ± 3.99</td>
</tr>
<tr>
<td>0.7%</td>
<td>78.17 ± 8.43</td>
<td>80.98 ± 6.42</td>
<td>79.62 ± 6.54</td>
<td>77.17 ± 5.46</td>
</tr>
<tr>
<td>0.9%</td>
<td>86.17 ± 7.06</td>
<td>89.00 ± 4.17</td>
<td>84.17 ± 5.21</td>
<td>81.17 ± 4.25</td>
</tr>
<tr>
<td>1%</td>
<td>92.17 ± 4.32</td>
<td>95.00 ± 2.45</td>
<td>90.00 ± 1.99</td>
<td>87.17 ± 1.25</td>
</tr>
<tr>
<td>1.2%</td>
<td>96.25 ± 2.31</td>
<td>99.25 ± 1.09</td>
<td>95.12 ± 1.99</td>
<td>91.12 ± 0.99</td>
</tr>
</tbody>
</table>

Means followed by same letter (or no letter) are not significant at the 0.05 probability level.
The KCl modified gel strength (KGS) and native water gel strengths (WGS) of SRC obtained from the samples of summer (KGS 510–625 g cm\(^{-2}\) and WGS 265–284 g cm\(^{-2}\)) and pre-monsoon months (KGS 495–590 g cm\(^{-2}\) and WGS 270–276 g cm\(^{-2}\)) were significantly higher (\(p = 0.003\)) than samples from winter (KGS 315–320 g cm\(^{-2}\) and WGS 185–218 g cm\(^{-2}\) and) and monsoon (KGS 350–365 g cm\(^{-2}\) and WGS 174–188 g cm\(^{-2}\)). However, these readings were much lower than SRC obtained from RDS of the same species farmed in Sri Lanka (KGS 600–1050 g cm\(^{-2}\) and WGS 245–770 g cm\(^{-2}\)) [35], in Vietnam (KGS 1190–1712 g cm\(^{-2}\) and WGS 245–557 g cm\(^{-2}\)) [31], (WGS 503–1105 g cm\(^{-2}\)) [33], in Indonesia (KGS 1022–1140 g cm\(^{-2}\)), in Philippines (KGS 1005–1224 g cm\(^{-2}\)) [31] and in Brazil (WGS 688–926 g cm\(^{-2}\)) [50] but the yield of SRC produced from dry K. alvarezii harvested from different seasons through the conventional process in this present study was 29.5–36.36%, with a high gel strength (WGS of 280–455 g cm\(^{-2}\) and KGS of 610–1010 g cm\(^{-2}\)); these readings were much comparable to literature reports [31,33,35,50]. Thus, lower yields and deterioration in quality were observed only in the SRC derived from the wet fibrous residue obtained after extraction of the bio-stimulant sap from K. alvarezii.
The viscosity of the SRC prepared from wet fibrous residue in the present study was in the range of 85–155 cP which compared to that of 35–87 cP for SRC obtained from the dry-weed process. Though the viscosity of the former was much higher than other reported values, the range of the latter was similar to that of SRC from K. alvarezii farmed elsewhere, i.e. Ohno et al. [31] reported viscosity ranging from 16 to 97 cP and Hung et al. [33] recorded 83–128 cP for carrageenan samples from the same species farmed in Vietnam and 27–72 cP in Sri Lanka [35]. The absorbencies at 1267–1235 cm$^{-1}$ (ester sulfate), 1047–1077 cm$^{-1}$ (for glycosidic linkage, C-O-C), 926–930 cm$^{-1}$ (3,6 anhydrogalactose) and 844–848 cm$^{-1}$ (galactose-sulfate) in the IR spectra showed that the SRC produced was kappa carrageenan [36] and showed no seasonal variations in the absorbencies in its IR spectra.

### 5. Conclusion

Fresh biomass of K. alvarezii yielded 2.0–2.3% bio-stimulant and 2.4–4.0% SRC with a 94–96% moisture. The economics of conversion of fresh seaweed into agricultural bio-stimulant and SRC through the MUZE process was better than the conversion of dry weed into SRC alone through the conventional method i.e. 1.0 t FS yielded a net profit of $224.14USD through the multi-stream process, whereas the dry-weed equivalent was $118.34USD when processed by the conventional method. The present investigation supported the practice that fresh K. alvarezii can be used as a raw material to co-produce an effective agricultural bio-stimulant as well as provide SRC with medium gel strength.

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### Author agreement statement

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us and we are aware of the submission of this manuscript to Algal Research for peer review.

### Statement of informed consent

No conflicts, informed consent, human or animal rights applicable.

### Conflict of interest statement

The authors have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

### Authors contributions

MS and AS engaged in conception and design of the experiment. MS engaged in analysis and interpretation of the data and drafting of the article. AS made critical revision of the article for important intellectual content.

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