



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



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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 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*

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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 AquAgri 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 t 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 8% 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:

$$\% \text{sulfate} = (W_2/W_1) \times 100 \times 0.4116,$$

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. KCl 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 media (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.

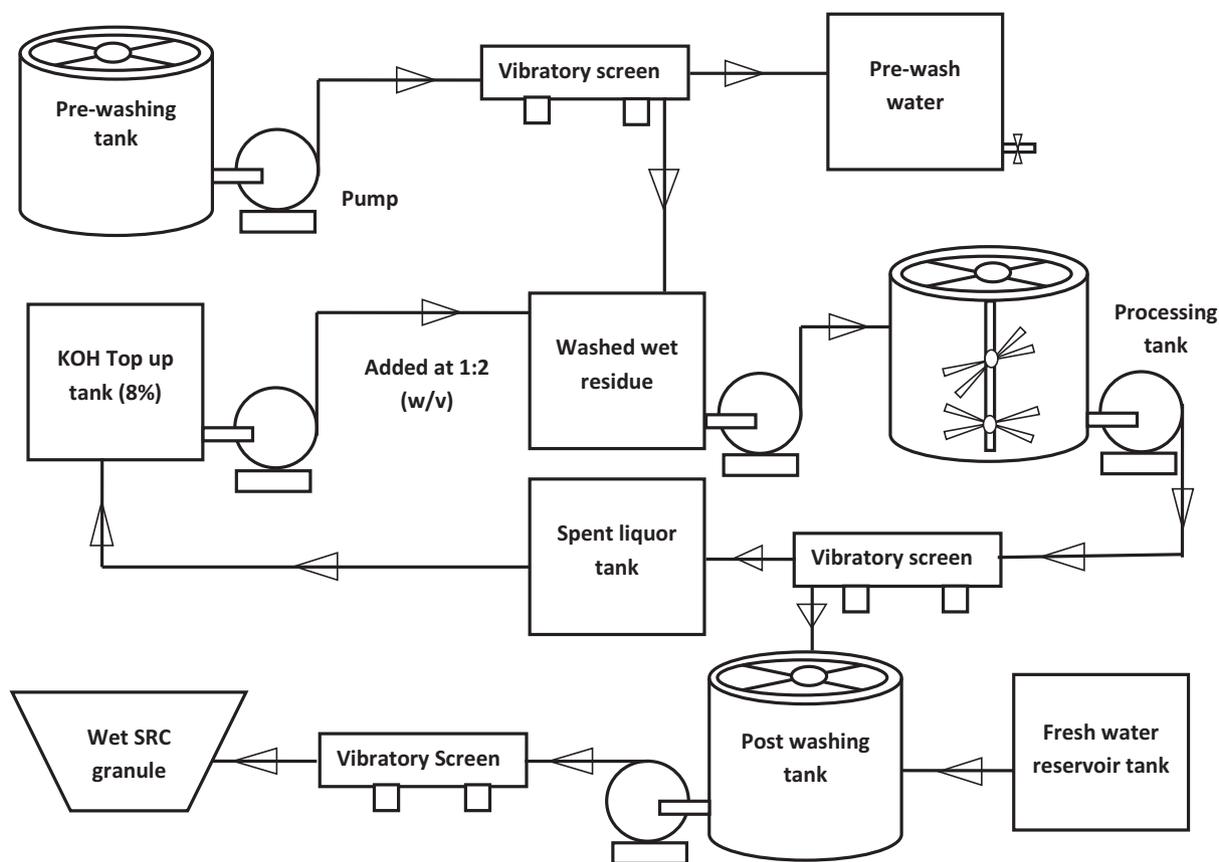


Fig. 1. Production flow chart of SRC from wet fibrous residue obtained after extraction of bio-stimulant sap from *K. alvarezii*.

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 $80 \pm 2^\circ\text{C}$ 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-

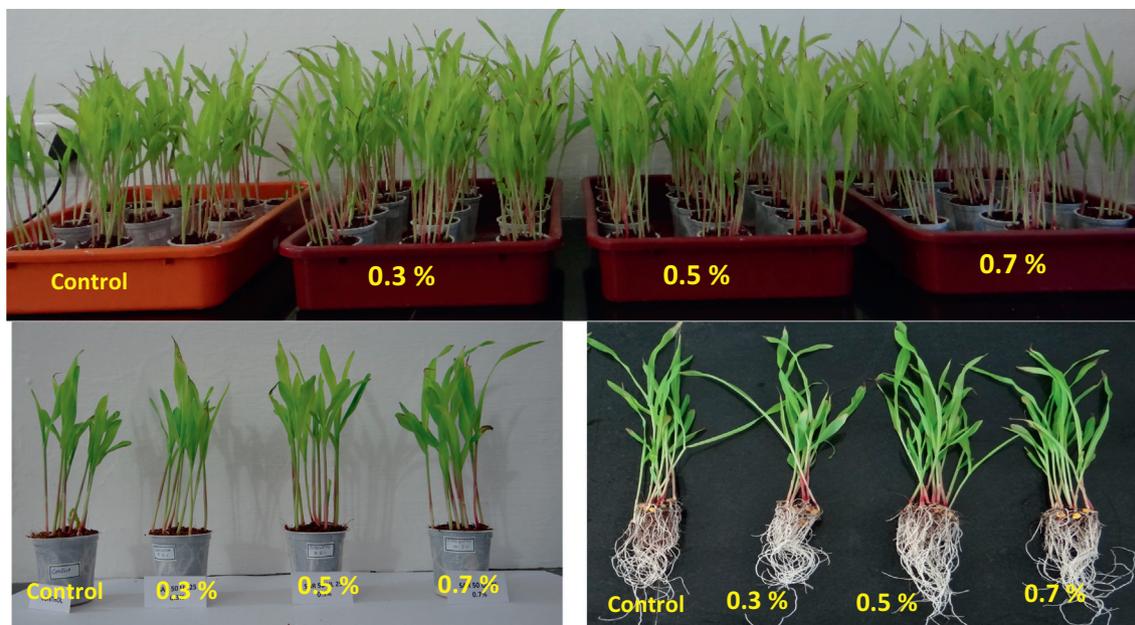


Fig. 2. Effect of bio-stimulant sap manufactured from the fresh biomass of *K. alvarezii* on the germination of maize seeds.

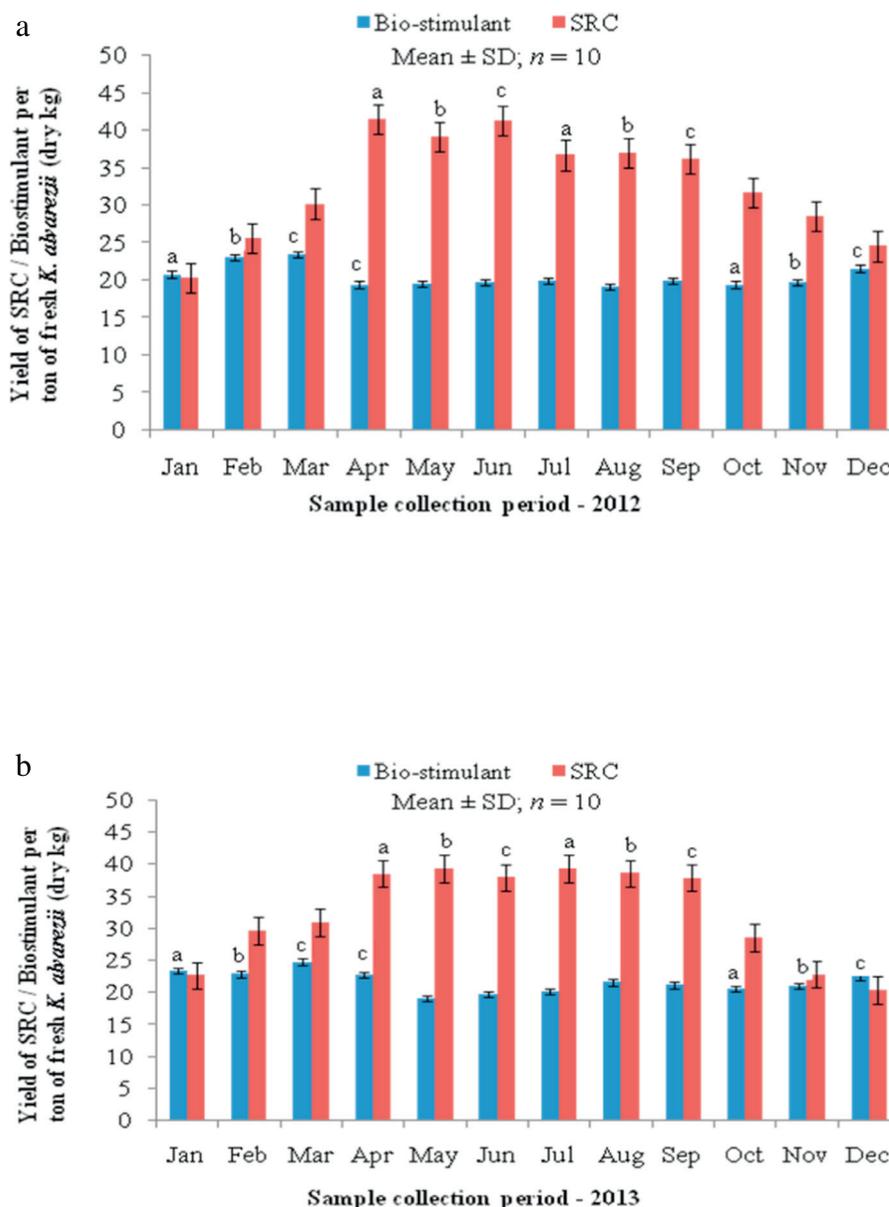


Fig. 3. a. Monthly variation in recovery of bio-stimulant sap and SRC (semi-refined carrageenan) obtained from 1.0 t fresh biomass of *K. alvarezii* farmed on the Tamil Nadu coast of India during Jan–Dec 2012 (means followed by same letter (or no letter) are not significant at the 0.05 probability level)

b. Monthly 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 Jan–Dec 2013. (means followed by same letter (or no letter) are not significant at the 0.05 probability level).

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 TLC 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 = (number of germinated seeds/ number of total seeds per treatment after 48 h \times 100)], germination energy [(GE % = (number of germinated seeds/ number of total seeds per treatment after 48 h \times 100)] and seedling vigor index [SVI = (seedling length (mm) \times germination percentage)] [39].

2.4. Statistical analysis

Data are presented as means \pm 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 $P < 0.05$.

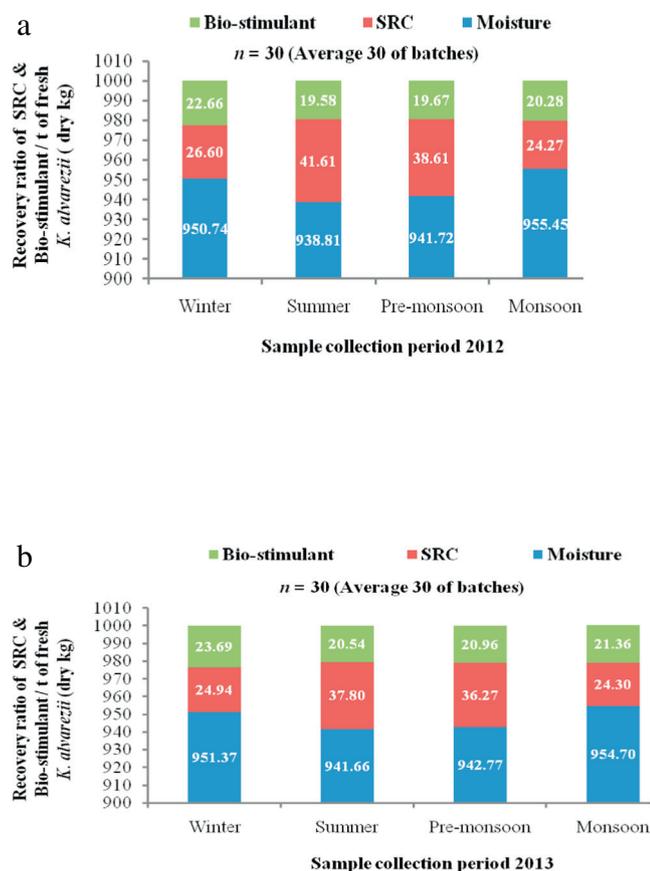


Fig. 4. a. Seasonal variation in recovery of bio-stimulant sap and SRC (semi-refined carrageenan) obtained from 1.0 t fresh biomass of *K. alvarezii* farmed on the Tamil Nadu coast of India during different seasons of 2012

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.

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 $0.81 \pm 0.05\%$, 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 (3.36–3.70%) 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 – $1.70 \pm 0.10\%$) than those from the winter - monsoon period (1.46 ± 0.11 – $1.55 \pm 0.08\%$). This was positively correlated to the TSS of the bio-stimulant sap and the same trend was observed with the nitrogen (180 ± 22 – 265 ± 28 ppm) and phosphorous contents (14 ± 2 – 38 ± 3 ppm) during the four seasons, over the two years studied. Calcium levels were reported as:

Table 1

Recovery ratio of agricultural bio-stimulant and semi-refined carrageenan co-manufactured from 1.0 t of fresh biomass of *K. alvarezii* (SD \pm ; $n = 30$).

Seasons	Bio-stimulant			Semi-refined carrageenan (SRC)		
	Bio-stimulant liquid per 1.0 t of fresh biomass (kg)	Total solid content of bio-stimulant (%)	Total bio-stimulant recoverable per 1.0 t of fresh biomass (dry kg)	Wet fibrous residue per 1.0 t of fresh biomass (kg)	SRC yield (%)	Total SRC per 1.0 t of fresh biomass (dry kg)
2012						
Winter	575 \pm 34.5	3.94 \pm 0.23	22.66 \pm 1.83 ^a	360 \pm 24.48	7.39 \pm 0.42	26.60 \pm 2.10
Summer	438 \pm 21.9	4.47 \pm 0.26	19.58 \pm 2.11 ^a	485 \pm 27.34	8.58 \pm 0.56	41.61 \pm 2.88 ^b
Pre-monsoon	465 \pm 28.3	4.23 \pm 0.19	19.67 \pm 2.70	463 \pm 26.40	8.34 \pm 0.60	38.61 \pm 0.300 ^c
Monsoon	576 \pm 38.0	3.52 \pm 0.20	20.28 \pm 2.55 ^{ab}	365 \pm 29.05	6.65 \pm 0.72	24.27 \pm 1.85 ^c
2013						
Winter	585 \pm 41.5	4.05 \pm 0.21	23.69 \pm 2.15 ^a	375 \pm 20.26	6.65 \pm 0.48	24.94 \pm 2.22
Summer	495 \pm 28.1	4.15 \pm 0.32	20.54 \pm 1.72 ^a	450 \pm 27.45	8.40 \pm 0.62	37.80 \pm 3.17 ^b
Pre-monsoon	510 \pm 39.7	4.11 \pm 0.25	20.96 \pm 1.68	445 \pm 28.84	8.15 \pm 0.78	36.27 \pm 2.69 ^c
Monsoon	605 \pm 47.7	3.53 \pm 0.27	21.36 \pm 1.74 ^{ab}	360 \pm 31.80	6.75 \pm 0.68	24.30 \pm 2.18 ^c

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

Table 2

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 \pm ; $n = 9$).

Physico-chemical parameters and PGRs	2012				2013			
	Winter	Summer	Pre-monsoon	Monsoon	Winter	Summer	Pre-monsoon	Monsoon
Total organic matter (g 100 g ⁻¹)	0.70 \pm 0.04	0.77 \pm 0.05	0.80 \pm 0.04	0.65 \pm 0.04	0.61 \pm 0.03	0.68 \pm 0.05	0.75 \pm 0.06	0.81 \pm 0.05
pH (1% solution)	6.81 \pm 0.02	6.78 \pm 0.08	7.20 \pm 0.10	6.68 \pm 0.11	6.88 \pm 0.05	6.80 \pm 0.03	6.91 \pm 0.05	6.50 \pm 0.05
Total soluble solid (g 100 g ⁻¹)	3.94 \pm 0.23	4.47 \pm 0.26 ^a	4.23 \pm 0.19 ^b	3.52 \pm 0.20 ^b	4.05 \pm 0.21	4.15 \pm 0.32 ^a	4.11 \pm 0.25 ^b	3.53 \pm 0.27
Total ash (g 100 g ⁻¹)	3.24 \pm 0.19	3.70 \pm 0.15	3.43 \pm 0.13	2.87 \pm 0.12	3.44 \pm 0.16	3.47 \pm 0.21	3.36 \pm 0.15	2.72 \pm 0.22
Specific gravity (g cm ⁻³)	1.03 \pm 0.002	1.03 \pm 0.003	1.03 \pm 0.003	1.03 \pm 0.001	1.03 \pm 0.002	1.0 \pm 0.003	1.03 \pm 0.002	1.03 \pm 0.003
Primary nutrients								
Nitrogen (N) (g 100 g ⁻¹)	185 \pm 45	225 \pm 22	252 \pm 40	180 \pm 22	208 \pm 33	258 \pm 37	265 \pm 28	204 \pm 14
Phosphorous (P) (g 100 g ⁻¹)	14 \pm 2.0	27 \pm 4.50	35 \pm 2.20	14 \pm 5.10	26 \pm 1.90	38 \pm 3.00	32 \pm 1.1	21 \pm 3.20
Potassium (K) (g 100 g ⁻¹)	1.46 \pm 0.11	1.69 \pm 0.08 ^a	1.70 \pm 0.10 ^b	1.50 \pm 0.08	1.55 \pm 0.08	1.67 \pm 0.07 ^a	1.62 \pm 0.05 ^b	1.54 \pm 0.09
Secondary nutrients								
Calcium (Ca) (mg kg ⁻¹)	266 \pm 45	412 \pm 82	320 \pm 55	157 \pm 37	210 \pm 34	380 \pm 65	227 \pm 88	190 \pm 44
Magnesium (Mg) (mg kg ⁻¹)	340 \pm 65	330 \pm 18	152 \pm 12	370 \pm 36	315 \pm 63	320 \pm 23	255 \pm 38	280 \pm 32
Sulphur (S) (mg kg ⁻¹)	212 \pm 20	265 \pm 23	145 \pm 15	222 \pm 11	95 \pm 21	137 \pm 24	330 \pm 63	105 \pm 14
Plant growth regulators (PGRs)								
Auxins (mg kg ⁻¹)	55.05 \pm 13.00 ^a	31.0 \pm 4.33 ^a	35.90 \pm 4.00	60.25 \pm 10.80 ^{ab}	68.20 \pm 9.50 ^a	30.18 \pm 6.50 ^a	25.25 \pm 8.50	70.20 \pm 16.20 ^{ab}
Cytokinins (mg kg ⁻¹)	28.99 \pm 15.90 ^b	22.03 \pm 11.50 ^b	20.50 \pm 16.20	45.65 \pm 8.80 ^{bc}	35.80 \pm 5.80 ^b	19.40 \pm 4.50 ^b	14.34 \pm 3.70	55.50 \pm 5.80 ^{bc}
Gibberellins (mg kg ⁻¹)	123.8 \pm 16.45 ^c	105.0 \pm 12.50 ^c	90.90 \pm 5.50	110.28 \pm 21.50 ^{ac}	140.70 \pm 7.50 ^c	52.44 \pm 8.00 ^c	62.00 \pm 7.50 ^{aa}	135.00 \pm 17.00 ^{ac}

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

210–266 ppm (winter), 380–412 ppm (summer), 227–320 ppm (pre-monsoon) and 157–190 ppm (monsoon); similarly, magnesium levels were 315–340 ppm (winter), 320–330 ppm (summer), 152–255 ppm (pre-monsoon) and 280–370 ppm (monsoon). (Table 2). The auxin content of the samples from the winter and monsoon months were 55.05 \pm 13–70.20 \pm 16 ppm which were higher than ($F = 104.73$; $p = 0.0001$) samples from the summer and pre-monsoon months (25.25 \pm 8.5–45.90 \pm 4.0 ppm) and same trend was observed in the case of cytokinin ($F = 23.07$; $p = 0.0013$) and gibberellic acids ($F = 21.20$; $p = 0.0017$) also.

Treatment at 0.5% and 0.7% gave > 90% of germination at 48 h reading whereas 0.3% treatment and control reached highest germination (> 90%) at 60 h reading. Since treatment at 0.5 & 0.7% reached > 90% GP at 48 h, and it was considered for calculating efficacy studies. The percentage of germination (PG) observed after 48 h is given in Table 3 and all treatments were significant ($P < 0.05$) over the control seeds. Seeds treated with 0.5% ranged between 92.17 \pm 3.00–96.85 \pm 3.44% and at 0.7% it was 89.17 \pm 3.42–92.70 \pm 4.14% whereas it was 70.33 \pm 5.51–77.33 \pm 2.24% in seeds treated with 0.3% bio-stimulant with 55.67 \pm 6.0–67.33 \pm 7.24% in the control seeds. The GE with treatments of 0.5 and 0.7% were 69.17 \pm 1.91–72.67 \pm 3.94% and 66.83 \pm 2.23–71.55 \pm 2.19% respectively and it was 55.17 \pm 3.33–58.17 \pm 4.88% for the 0.3% treatment and in the control, it was 41.83 \pm 4.41–46.00 \pm 2.71%. The seed vigor index (SVI) of the control seeds was between 10,768.69 and 13,132.35 and highest readings were reported in seeds treated with 0.5% (19,741.32 to 21,500.70) followed by 0.7% (i.e. 18,198.18 to 20,179.80) and 0.3% (13,985.00 to 16,457.45). The PG, GE and SVI were observed to be significantly higher in those seeds treated with 0.5 and 0.7% ($P < 0.05$) and the results showed that only the concentration of the bio-stimulant sap influenced the germination, regardless of the season of its production.

3.3. Quality of SRC

The quality of the SRC produced from the wet fibrous residue after extraction of the sap intended for applications as a bio-stimulant is given in Table 3. The moisture content of the SRC ranged between 4.45 \pm 0.37–11.21 \pm 0.75% and pH was 9.1–9.8. The KCl salt level in all SRC samples tested was between 1.12 and 1.97% and the ash content was 21.76–26.13%. The average sulfate content of the SRC was significantly higher in samples from the winter (21.43 \pm 0.56%) and monsoon months (22.50 \pm 0.63%), as compared with those from the summer

(18.57 \pm 1.10%) and pre-monsoon months (17.65 \pm 0.95%) ($F = 34.79$; $p = 0.0036$) and it was positively correlated to ash content ($r = 0.912$; $p = 0.0033$) and negatively correlated ($r = -0.766$; $p = 0.001$) to the content of 3,6-AG (winter-monsoon 27.38 \pm 0.17–29.01 \pm 0.39%; summer and pre-monsoon 31.55 \pm 0.81–32.54 \pm 0.68%). Significant differences in the native water gel strength (WGS) and the KCl modified gel strength (KGS) of SRC samples were found higher ($F = 70.14$; $p = 0.0003$) in the summer and pre-monsoon months' samples (i.e. WGS 265.72–284.22 g cm⁻²; KGS 495.24–625.55 g cm⁻²) rather than the winter and monsoon seasons (i.e. WGS 174.66–218.85 g cm⁻²; KGS 315.20–365.40 g cm⁻²). The viscosity readings of the SRC were 105.60–155.20 cP (winter), 85.54–143.18 cP (summer), 90.32–135.72 cP (pre-monsoon) and 127.24–135.90 cP (monsoon) for both years studied (Table 4). In the IR spectra, absorbance at 1235–1267 cm⁻¹ correlated to ester sulfate and absorbance at 1047–1077 cm⁻¹ was assigned to glycosidic linkages, the presence of 3,6 anhydrogalactose was identified from the absorbance occurring between the 926–930 cm⁻¹ bands and those at 844–848 cm⁻¹ were assigned to galactose-4-sulfate [30]. Very strong, strong and medium indicated the relative intensities in the IR spectrum (Table 5). The total plate counts of the SRC samples were 2500 and yeast and moulds were < 100 (25 g), no pathogenic microbes such as *Shigella*, *Salmonella* and *E. coli* were found. The yield of SRC produced from dry *K. alvarezii* over different seasons by the conventional process was 29.5–36.36% with WGS of 280–455 g cm⁻² and KGS of 610–1010 g cm⁻² and viscosity ranged between 35 and 67 cP (Table 6). The economic evaluation of MUZE processing of fresh seaweed for making agricultural bio-stimulant and SRC versus processing of dry weed through conventional methods to produce only SRC was calculated, i.e. 1.0 t FS yielded a net profit of \$ 224.14USD through multi-stream processing, whereas its dry-weed equivalent produced only \$ 118.34USD (Table 7).

4. Discussion

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

Table 3
Effect of bio-stimulant produced from *K. alvarezii* farmed in different seasons of 2012 and 2013 on the germination of maize (SD ± ; n = 9).

	2012				2013			
	Winter	Summer	Pre-monsoon	Monsoon	Winter	Summer	Pre-monsoon	Monsoon
Germination percentage (%)								
Control	61.83 ± 7.31	55.67 ± 6.00	67.33 ± 7.24	62.75 ± 5.91	63.61 ± 6.51	57.40 ± 7.19	66.55 ± 6.15	59.80 ± 6.33
0.3%	74.30 ± 8.23	77.33 ± 2.24	70.33 ± 5.51	73.50 ± 4.33	73.55 ± 7.77	73.64 ± 6.60	73.12 ± 6.00	75.15 ± 6.03
0.5%	93.67 ± 3.20 ^a	94.00 ± 1.44 ^b	94.00 ± 2.37 ^c	92.17 ± 3.0 ^d	94.54 ± 4.32 ^{ab}	95.12 ± 3.44 ^{bs}	96.85 ± 3.44 ^{bc}	93.30 ± 4.29 ^{dc}
0.7%	90.33 ± 4.94 ^{ab}	91.17 ± 3.46 ^{bc}	90.83 ± 3.82 ^{bc}	89.17 ± 3.42 ^{dc}	90.09 ± 4.82 ^a	92.27 ± 3.46 ^b	92.70 ± 4.14 ^c	90.90 ± 4.43 ^d
Germination energy								
Control	46.00 ± 2.71	43.67 ± 1.97	43.17 ± 2.51	41.83 ± 4.41	45.08 ± 3.80	42.88 ± 2.69	44.41 ± 1.97	42.38 ± 4.11
0.3%	55.67 ± 5.13	58.17 ± 4.88	53.00 ± 3.04	56.33 ± 2.69	56.33 ± 6.05	56.70 ± 5.00	55.17 ± 3.33	57.71 ± 3.07
0.5%	70.50 ± 1.91 ^a	71.67 ± 2.03 ^b	72.67 ± 3.94 ^c	69.17 ± 1.91 ^d	72.40 ± 2.22 ^{ab}	70.12 ± 3.60 ^{bc}	69.99 ± 4.12 ^{bc}	71.14 ± 2.13 ^{dc}
0.7%	68.00 ± 3.09 ^{ab}	68.50 ± 0.87 ^{bc}	71.33 ± 2.36 ^{bc}	66.83 ± 2.23 ^{dc}	67.32 ± 3.11 ^a	71.55 ± 2.19 ^b	70.23 ± 3.26 ^c	70.01 ± 3.14 ^d
Seedling vigor index								
Control	11,526.18 ± 1309.64	10,768.69 ± 1137.18	13,132.35 ± 1563.43	12,127.50 ± 678.99	11,767.85 ± 1059.11	10,733.80 ± 966.04	12,711.05 ± 1525.33	11,182.60 ± 1266.99
0.3%	13,985.00 ± 2066.20 ^{ab}	16,457.45 ± 1117.74 ^{ab}	14,967.78 ± 1613.26 ^{ab}	14,377.37 ± 1734.55 ^{ab}	14,342.25 ± 1147.38 ^{ab}	14,749.80 ± 1297.98 ^{ab}	14,185.28 ± 1702.23 ^{aa}	14,954.85 ± 1645.03 ^{aa}
0.5%	20,028.32 ± 188.41 ^a	20,492.12 ± 734.63 ^b	19,917.79 ± 1323.73 ^c	19,741.32 ± 1674.90 ^d	20,987.88 ± 931.86 ^{bc}	21,306.88 ± 779.83 ^{bc}	21,500.70 ± 1419.05 ^{bc}	20,293.30 ± 1126.28 ^{dc}
0.7%	18,837.16 ± 1103.44 ^{ab}	19,972.24 ± 1097.62 ^{bc}	18,693.81 ± 552.02 ^{bc}	18,718.44 ± 1426.93 ^{dc}	18,198.18 ± 1182.88 ^a	18,638.54 ± 827.55 ^b	18,447.30 ± 1217.52 ^c	20,179.80 ± 1083.66 ^d

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

summer - pre-monsoon months rather than those from the winter - monsoon months. Yield of wet fibrous residue from samples of summer and pre-monsoon was higher than other two seasons, and this was reflected in higher yield of SRC in those seasons. However, the reverse was true for the recovery of bio-stimulant sap, i.e. winter - monsoon months yielded 20.97% more recovery than the summer and pre-monsoon seasons (Fig. 3a & b).

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 *Ascophyllum 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 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 [35], 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 4Quality of SRC manufactured from wet fibrous residue of *K. alvarezii* after extraction of biostimulant sap (SD \pm ; n = 30).

Parameters	2012				2013			
	Winter	Summer	Pre-monsoon	Monsoon	Winter	Summer	Pre-monsoon	Monsoon
Moisture (%)	4.45 \pm 0.37	7.86 \pm 0.48	8.81 \pm 0.70	10.04 \pm 0.96	6.5 \pm 0.57	8.50 \pm 0.86	7.56 \pm 0.68	11.21 \pm 0.75
pH (1% soln.)	9.7 \pm 0.21	9.8 \pm 0.12	9.8 \pm 0.15	9.5 \pm 0.20	9.6 \pm 0.22	9.5 \pm 0.27	9.1 \pm 0.12	9.7 \pm 0.17
KCl (%)	1.29 \pm 0.58	1.45 \pm 0.53	1.12 \pm 0.32	1.97 \pm 0.40	1.21 \pm 0.37	1.32 \pm 0.44	1.25 \pm 0.29	1.40 \pm 0.51
Sulfate (%)	21.43 \pm 0.56	18.57 \pm 1.10	17.65 \pm 0.95	22.50 \pm 0.63	21.95 \pm 0.48	19.61 \pm 1.27	20.45 \pm 0.85	22.44 \pm 0.47
3,6AG (%)	28.19 \pm 0.45	32.41 \pm 0.60	31.55 \pm 0.81	27.70 \pm 0.66	29.01 \pm 0.39	32.18 \pm 0.97	32.54 \pm 0.68	27.38 \pm 0.17
Total ash (%)	22.82 \pm 1.37	21.76 \pm 1.10	22.45 \pm 0.76	26.13 \pm 0.96	24.90 \pm 1.50	22.67 \pm 0.88	22.81 \pm 0.54	25.74 \pm 1.22
Acid insoluble ash (%)	1.88 \pm 0.16	1.56 \pm 0.16	1.70 \pm 0.20	1.66 \pm 0.22	1.82 \pm 0.12	1.74 \pm 0.23	1.11 \pm 0.12	1.90 \pm 0.15
Native water gel strength (g cm ⁻²)	185.15 \pm 17.7	284.22 \pm 23.5 ^a	276.70 \pm 25.0 ^b	174.66 \pm 25.8	218.85 \pm 20.5	265.72 \pm 18.8 ^{bc}	270.86 \pm 18.5 ^{ac}	188.35 \pm 20.0
KCl modified gel strength (g cm ⁻²)	320.52 \pm 25.5	510.32 \pm 27.4 ^b	495.24 \pm 18.7 ^c	365.40 \pm 27.4	315.2 \pm 22.4	625.55 \pm 33.0 ^{cm3}	590.50 \pm 32.1 ^{dc}	350.05 \pm 27.3
Viscosity (cP)	105.60 \pm 8.5	85.54 \pm 6.0	90.32 \pm 6.4	127.24 \pm 5.5	155.20 \pm 4.8	143.18 \pm 7.0	135.72 \pm 5.7	135.90 \pm 5.8
Total plate count (TPC)	< 2500	< 2500	< 2500	< 2500	< 2500	< 2500	< 2500	< 2500
Yeast and Moulds	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100
<i>Salmonella</i> & <i>Shigella</i>	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
<i>Escherichia coli</i>	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

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

Table 5Wave numbers in FT-IR spectra of SRC manufactured from wet fibrous residue after extraction of bio-stimulant sap from *K. alvarezii* farmed in different seasons of 2012 and 2013.

Functional groups	Wave numbers (SRC produced in 2012)				Wave numbers (SRC produced in 2013)			
	Winter	Summer	Pre-monsoon	Monsoon	Winter	Summer	Pre-monsoon	Monsoon
Ester sulfate	1267 vs	1235 vs	1258 vs	1250 vs	1261 vs	1258 vs	1259 vs	1237 vs
Glycosidic linkage	1074 vs	1047 vs	1050 vs	1076 vs	1065 vs	1060 vs	1077 vs	1058 vs
3,6-anhydro-D-galactose	929 s	927 s	930 s	930 s	928 s	926 s	928 s	928 s
D-galactose-4-sulphate	846 m	847 m	848 m	848 m	845 m	846 m	844 m	844 m

Note: vs = very strong; s = strong; m = medium.

Table 6Quality of SRC manufactured from dry *K. alvarezii* (whole weed) by conventional method (SD \pm ; n = 30)*.

Parameters	2012				2013			
	Winter	Summer	Pre-monsoon	Monsoon	Winter	Summer	Pre-monsoon	Monsoon
SRC yield (%)	29.65 \pm 2.10	36.36 \pm 1.80	35.10 \pm 2.12	31.75 \pm 0.96	32.60 \pm 2.66	35.18 \pm 1.15	36.05 \pm 2.22	33.13 \pm 1.54
Moisture (%)	7.7 \pm 0.55	7.6 \pm 0.25	7.65 \pm 0.31	8.5 \pm 0.96	8.5 \pm 1.60	6.4 \pm 0.58	6.76 \pm 0.77	8.0 \pm 1.50
pH (1% soln.)	9.8 \pm 0.18	10.2 \pm 0.15	10.1 \pm 0.18	9.9 \pm 0.33	10.0 \pm 0.30	9.7 \pm 0.70	10.0 \pm 0.12	9.9 \pm 0.16
Sulfate (%)	18.80 \pm 1.36	17.44 \pm 1.23	18.0 \pm 1.95	20.81 \pm 0.86	17.50 \pm 0.77	16.90 \pm 1.14	16.05 \pm 1.60	19.25 \pm 0.78
Total ash (%)	23.85 \pm 1.40	22.08 \pm 1.55	23.60 \pm 1.10	24.65 \pm 1.33	24.12 \pm 1.48	21.60 \pm 1.15	23.05 \pm 1.11	26.75 \pm 1.19
Acid insoluble ash (%)	0.54 \pm 0.11	0.70 \pm 0.05	0.72 \pm 0.06	1.20 \pm 0.44	0.65 \pm 0.10	0.75 \pm 0.33	1.0 \pm 0.12	0.66 \pm 0.7
Native water gel strength (g cm ⁻²)	330.55 \pm 22.0	410.25 \pm 35.0 ^a	375.00 \pm 18.5 ^b	360.05 \pm 25.2	280.25 \pm 43.0	455.25 \pm 28.5 ^{ac}	420.20 \pm 20.0 ^{bc}	315.50 \pm 33.20
KCl modified gel strength (g cm ⁻²)	685.42 \pm 4.2	950.52 \pm 38.5 ^b	1010.65 \pm 25.2 ^c	750.25 \pm 30 ^{aa}	630.00 \pm 15.0	980.25 \pm 45.5 ^{cm3}	915.20 \pm 33.2 ^{dc}	610.5 \pm 22.0
Viscosity (cP)	48.25 \pm 5.5	37.50 \pm 5.0	35.50 \pm 4.5	63.25 \pm 7.0	55.00 \pm 11.0	38.25 \pm 6.5	42.20 \pm 6.0	87.70 \pm 8.0
Total plate count (TPC)	< 2500	< 2500	< 2500	< 2500	< 2500	< 2500	< 2500	< 2500
Yeast and moulds	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100
<i>Salmonella</i> & <i>Shigella</i>	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
<i>Escherichia coli</i>	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

* Data from SRC production log sheet of AquAgri (P) Ltd. during 2012–13. 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 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*.

Table 7
Economic valuation of multi-stream processing of fresh-weed Vs its equivalent dry-weed of *K. alvarezii*.

Investment and return	Multi stream process of fresh weed		Conventional process of dry-weed (C)
	Recovery of bio-stimulant (A)	Recovery of SRC (B)	
Investment			
Cost of 1 t FW or its equivalent dry-weed (US\$)	56.82		56.82
Freight cost of raw material from farming site to processing unit (US\$)	45.45		4.55
Process cost of bio-stimulant sap at 0.1 US\$ l ⁻¹	57.5		
Process cost of SRC from wet residue at 0.74 USD kg ⁻¹ (26.25 * 0.74)		19.42	
Process cost of SRC from dry-weed at 0.37 US\$ kg ⁻¹ (33.5 * 0.37)			21.29
Preservative cost for 575 l of bio-stimulant sap	17.42		
Cost of packaging material and label for 575 l of bio-stimulant sap	15.25		
Total conversion cost for 1 t of fresh or its equivalent dry-weed (US\$)	192.44	19.42	82.66
Total investment (A + B) & C (US\$)	211.86		82.66
Return			
Recovery of total bio-stimulant sap from 1.0 t of fresh weed (l)	575		
Total SRC recovered from 1 t of fresh or its equivalent dry-weed (dry kg)		26.25	33.50
Total sale value of finished goods (US\$)	304.75	131.25	201
Total sale value (US\$)	418		201.0
Net return through processing of 1 t fresh weed or its equivalent dry-weed (US\$)	206.14		118.34

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-4-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. 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.

References

- [1] P. Calvo, L. Nelson, J.W. Kloepper, Agricultural uses of plant bio-stimulants, *Plant Soil* 383 (2014) 3–41.
- [2] M.J. Van Oosten, O. Pepe, S. De Pascale, S. Stilletti, A. Maggio, The role bio-stimulants and bioeffectors as alleviators of abiotic stress in crop plants, *Chem. Biol. Technol. Agric.* 4 (2017) 1–12.
- [3] Intenet-1 Bio-stimulant market – Global trends & forecasts to 2021, www.marketsandmarkets.com, Accessed date: 12 November 2016.
- [4] I.C. Neish, Feasibility assessment for a Zanzibar MUZE Seaweed Processing Facility (ZanMUZE), Report Submitted for UNIDO Project No 1308 3, Building Seaweed Processing Capacities in Zanzibar and Pemba: Creating Value for the Poor, 2013.
- [5] W. Khan, U.P. Rayirath, S. Subramanian, M.N. Jithesh, P. Rayorath, D.M. Hodges, A.T. Critchley, J.S. Craigie, J. Norrie, B. Prithiviraj, Seaweed extract as bio-stimulants of plant growth and development, *J. Plant Growth Regul.* 28 (2009) 386–399.
- [6] J.S. Craigie, Seaweed extract stimuli in plant science and agriculture, *J. Appl. Phycol.* 23 (2011) 371–393.
- [7] R.F. Milton, Liquid seaweed as a fertilizer, *Proc. Int. Seaweed Symp.* 4 1964, pp. 428–431.
- [8] H.S.S. Sharma, C. Fleming, C. Selby, J.R. Rao, T. Martin, Plant bio-stimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses, *J. Appl. Phycol.* 26 (2014) 465–490.
- [9] J. Norrie, J.P. Keathley, Benefits of *Ascophyllum nodosum* marine-plant extract applications to "Thompson seedless" grape production, *Acta Hort.* (727) (2006) 243–247.
- [10] H.S.S. Sharma, G. Lyons, C. McRoberts, D. McCall, E. Carmichael, F. Andrewa, R. Swan, R. McCormack, R. Mellon, Bio-stimulant activity of brown seaweed species from Strangford lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (*Vigna mungo* L.) and pak choi (*Brassica rapa chinensis* L.),

- J. Appl. Phycol. 24 (2012) 1081–1091.
- [11] S.S. Rathore, D.R. Chaudhary, G.N. Boricha, A. Ghosh, B.P. Bhatt, S.T. Zodape, J.S. Patolia, Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions, S. Afr. J. Bot. 75 (2009) 351–355.
- [12] S.T. Zodape, S. Mukhopadhyay, K. Eswaran, M.P. Reddy, J. Chikara, Enhanced yield and nutritional quality in green gram (*Phaseolus radiata* L.) treated with seaweed (*Kappaphycus alvarezii*) extract, J. Sci. Ind. Res. India 69 (2010) 468–471.
- [13] M.T. Shah, S.T. Zodape, D.R. Chaudhary, K. Eswaran, J. Chikara, Seaweed sap as an alternative to liquid fertilizer for yield and quality improvement of wheat, J. Plant Nutr. 36 (2013) 192–200.
- [14] K. Karthikeyan, M. Shanmugam, The effect of potassium rich bio-stimulant from seaweed *Kappaphycus alvarezii* on yield and quality of cane and cane juice of sugarcane var. Co 86032 under plantation and ratoon crops, J. Appl. Phycol. 29 (2017) 3245–3252.
- [15] K. Karthikeyan, M. Shanmugam, Enhanced yield and quality in some banana varieties applied with commercially manufactured bio-stimulant Aquasap from sea plant *Kappaphycus alvarezii*, J. Agric. Sci. Technol. B. 4 (2014) 621–631.
- [16] K. Karthikeyan, M. Shanmugam, Development of a protocol for the application of commercial bio-stimulant manufactured from *Kappaphycus alvarezii* in selected vegetable crops, J. Exp. Biol. Agric. Sci. 4 (2016) 92–102.
- [17] S.T. Zodape, V.J. Kawarkhe, J.S. Patolia, A.D. Warade, Effect of liquid seaweed fertilizer on yield and quality of okra (*Abelmoschus esculentus* L.), J. Sci. Ind. Res. 67 (2008) 1115–1117.
- [18] S.T. Zodape, M. Soumita, K. Eswaran, M.P. Reddy, J. Chikara, Enhanced yield and nutritional quality in green gram (*Phaseolus radiata* L.) treated with seaweed (*Kappaphycus alvarezii*) extract, J. Sci. Ind. Res. 69 (2008) 468–471.
- [19] D. Mondal, A. Ghosh, K. Prasad, S. Singh, N. Bhatt, S.T. Zodape, J.P. Chaudhary, J. Chaudhari, P.B. Chatterjee, A. Seth, P.K. Ghosh, Elimination of gibberellins from *Kappaphycus alvarezii* seaweed sap foliar spray enhances corn Stover production without compromising the grain yield advantage, Plant Growth Regul. 75 (2015) 657–666.
- [20] K. Prasad, A.K. Dass, M.D. Oza, H. Brahmabhatt, A.K. Siddhanta, R. Meena, K. Eswaran, M.R. Rajyaguru, P.K. Ghosh, Detection and quantification of some plant growth regulators in a seaweed-based foliar spray employing a mass spectrometric technique sans chromatographic separation, J. Agric. Food Chem. 58 (2010) 4594–4601.
- [21] W.A. Stirk, J.V. Staden, Seaweed products as bio-stimulants in agriculture, in: A.T. Critchley, M. Ohno, B.D. Largo (Eds.), World Seaweed Resources [DVD-ROM], ETI Information Services Ltd, Univ, Amsterdam, 2006, pp. 80–84 (ISBN: 9075000).
- [22] D. Battacharyya, M.Z. Babgohari, P. Rathore, B. Prithiviraj, Seaweed extracts as bio-stimulants in horticulture, Sci. Hortic. 196 (2015) 39–48.
- [23] R. Hoffmann, M. Gidley, D. Cooke, W. Frith, Effect of isolation procedures on the molecular composition and physical properties of *Eucheuma cottonii* carrageenan, Food Hydrocoll. 9 (1995) 281–289.
- [24] G.O. Philips, The Chemical Identification of PNG Carrageenan, Newtech Innovation Centre, Croesnewydd Hall, Wrexham, LL13, 7YP, Wales, UK, 2002.
- [25] A.S. Mehta, K.H. Mody, I. Anita, P.K. Ghosh, Preparation of semi-refined k-carrageenan: recycling of alkali solution and recovery of alkali from spent liquor, Ind. J. Chem. Tech. 15 (2008) 45–52.
- [26] S. Abhiram, M. Shanmugam, Seaweeds as agricultural crops in India: new vistas, in: J.C. Dagar, P.C. Sharma, D.K. Sharma, A.K. Singh (Eds.), Innovative Saline Agriculture, Springer India, New Delhi, 2016, pp. 441–473.
- [27] B.C. Featonby-Smith, J.V. Staden, Identification and seasonal variation of endogenous cytokinins in *Ecklonia maxima* (Osbeck) Papenfuss, Bot. Mar. 27 (1984) 527–530.
- [28] G. Blunden, P.F. Morse, I. Mathe, J. Hohmann, A.T. Critchley, S. Morrell, Betaine yields from marine algal species utilized in the preparation of seaweed extracts used in agriculture, Nat. Prod. Commun. 5 (2010) 581–585.
- [29] H.B. Papenfuss, (Only one 'S' is there in ref), W.A. Stirk, J.F. Finnie, J.V. Staden, seasonal variation in the polyamines of *Ecklonia maxima*, Bot. Mar. 55 (2012) 539–546.
- [30] S.M. Mohy El-Din, Utilization of seaweed extracts as biofertilizers to stimulate the growth of wheat seedlings, Egypt, J. Exp. Biol. 11 (2015) 31–39.
- [31] M. Ohno, H.O. Nang, S. Hirase, Cultivation and carrageenan yield and quality of *Kappaphycus alvarezii* in the waters of Vietnam, J. Appl. Phycol. 8 (1996) 431–437.
- [32] A.Q. Hurtado, R.F. Agbayani, R. Sanares, M.T.R. Castro-Mallare, The seasonality and economic feasibility of cultivating *Kappaphycus alvarezii* in Panagatan Cays, Caluya, Antique, Philippines, Aquaculture 199 (2001) 295–310.
- [33] L.D. Hung, K. Hori, H.Q. Nang, T. Kha, L.T. Hoa, Seasonal changes in growth rate, carrageenan yield and lectin content in the red alga *Kappaphycus alvarezii* cultivated in Camranh Bay, Vietnam, J. Appl. Phycol. 21 (2009) 265–272.
- [34] K. Eswaran, P.K. Ghosh, A.K. Siddhanta, J.S. Patolia, C. Periyasamy, A.S. Mehta, K. H. Mody, B.K. Ramavat, K. Prasad, M.R. Rajyaguru, C.R.K. Reddy, J.B. Pandya, A. Tewari, Integrated method for production of carrageenan and liquid fertilizer from fresh seaweeds. US patent 6893479 B2, filed August 19, 2002. <http://www.google.co.in/patents/US6893479> (2005).
- [35] M. Shanmugam, K. Sivaram, E. Rajeev, V. Pahalawattarachchi, P.N. Chandraratne, J.M. Asoka, S. Abhiram, Successful establishment of commercial farming of carrageenophyte *Kappaphycus alvarezii* Doty (Doty) in Sri Lanka: economics of farming and quality of dry seaweed, J. Appl. Phycol. 29 (2017) 3015–3027.
- [36] J. Moses, R. Anandhakumar, M. Shanmugam, Effect of alkaline treatment on the sulfate content and quality of semi-refined carrageenan prepared from seaweed *Kappaphycus alvarezii* Doty (Doty) farmed in Indian waters, Afr. J. Biotechnol. 14 (2015) 1584–1589.
- [37] W. Yaphe, G.P. Arsenaault, Improved resorcinol reagent for the determination of fructose and 3,6-anhydrogalactose in polysaccharide, Anal. Biochem. 13 (1965) 143–148.
- [38] S. Cruchaga, S.A. Echeita, A. Aladuenca, J. Garciapena, N. Frias, M.A. Usera, Antimicrobial resistance in *Salmonella* from human, food and animals in Spain in 1998, J. Antimicrob. Chemother. 47 (2001) 315–321.
- [39] R.M.H. Herrera, F.S. Ruvalcaba, M.A. Ruiz-Lopez, J. Norrie, G.H. Carmona, Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.), J. Appl. Phycol. 26 (2014) 619–628.
- [40] K.M. Kim, Seasonal variation of seaweed components and novel biological function of fucoidan extracted from brown algae in Quebec (doctoral dissertation, Université Laval). Kinetin on potato yields, J. Sci. Food Agric. 28 (2012) 121–125.
- [41] H.M. Khairy, S.M. El-Shafay, Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt, Oceanologia 55 (2013) 435–452.
- [42] G. Sudha, G.A. Ravishankar, Involvement and interaction of various signaling compounds on the plant metabolic events during defense response, resistance to stress factors, formation of secondary metabolites and their molecular aspects, Plant Cell Tissue Organ Cult. 71 (2002) 181–212.
- [43] A.N. Moschou, K.A. Paschalidis, I.D. Delis, A.H. Andriopoulou, G.D. Lagiotis, D.I. Yakoumakis, K.A. Roubelakis-Angelakis, Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H₂O₂ signatures that direct tolerance responses in tobacco, Plant Cell 20 (2008) 1708–1724.
- [44] A. Guzmán-Urrióstegui, P. Garcia-Jimenez, F.D. Marián, D. Robledo, R.R. Robaina, Polyamines influence maturation in reproductive structures of *Gracilaria cornea* (Gracilariales, Rhodophyta), J. Phycol. 38 (2002) 1169–1175.
- [45] D. Valero, D. Martínez-Romero, M. Serrano, The role of polyamines in the improvement of the shelf-life of fruit, Trends Food Sci. Technol. 13 (2002) 228–234.
- [46] N. Demir, B. Dural, K. Yildirim, Effect of seaweed suspensions on seed germination of tomato, pepper and aubergine, J. Biol. Sci. 6 (2006) 1130–1133.
- [47] G.J. Christobel, Effect of seaweed (*Sargassum wightii* L.) on the germination and growth of green gram (*Phaseolus aureus*, L.), J. Basic Appl. Biol. 2 (2008) 105–108.
- [48] P.C. Mercer, R.B. Copeland, H.S.S. Sharma, J. Bingham, Seaweed extracts as possible agents in improving the emergence of barley, oats and maize in Northern Ireland, Adv. Anim. Biosci. 1 (2010) 332.
- [49] A. Iskandar, R. Syam, D.D. Trijuno, D. Rahmi, Content of carrageenan, chlorophyll a and carotenoid of *Kappaphycus alvarezii* cultivated in different seawater depth Laikang Village, district of Mangarabombang, Takalkar regency, J. Appl. Biotechnol. 2 (2013) 1–9.
- [50] L. Hayashi, E.J. Paula, F. Chow, Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the sub-tropical waters of Sao Paulo state, Brazil, J. Appl. Phycol. 19 (2007) 505–511.
- [51] H.G. Goés, R.P. Reis, Temporal variation of the growth, carrageenan yield and quality of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) cultivated at Sepetiba Bay, southeastern Brazilian coast, J. Appl. Phycol. 24 (2012) 173–180.
- [52] J. Munoz, Y. Freile-Pelegrín, D. Robledo, Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatán, México, Aquaculture 239 (2004) 161–177.