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Chaetomorpha antennina (Bory) Kützing derived seaweed liquid fertilizers as prospective bio-stimulant for *Lycopersicon esculentum* (Mill)

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ABSTRACT

Plant nutrition serve as critical factors that influences plant growth and yield. Global fertilizer production and application is growing in surplus amounts, increasing the agricultural expenses and damaging the environment. Organic fertilizers, obtained from natural sources, encompass all essential plant nutrients. Application of aqueous seaweed extracts are regarded as potential plant biostimulant agents. The liquid seaweed extract of green seaweed *Chaetomorpha antennina* (CA-LSE) were evaluated for their biostimulant activity on seed germination and growth of tomato. The CA-LSEs were able to stimulate early emergence of tomato seeds, in addition to increasing their germination percentage and energy. They also exerted a positive influence on the vegetative growth, resulting in increased plant height, leaf-branch number and yield. The CA-LSEs were influence the plant's biochemical profile and displayed a linear increase in pigment contents (chlorophyll a & b and carotenoids), total soluble solids (TSS), phenols as well as ascorbic acid contents. The bio-stimulant potential was attributed to the elements present in CA-LSEs, elucidated by EDX analysis that revealed the presence of six elements (O, Na, Mg, S, Cl and Ca), that were essentially plant nutrients. These results support the bio-stimulant potential of *C. antennina*, which can be applied as a prospective bio-fertilizer that is economic, renewable, efficient and eco-friendly, and also can be regarded as a potential catalyst for the betterment of sustainable agricultural food production.

1. Introduction

Plants are immobile organisms henceforth; they cannot avoid extreme environmental conditions. The biotic and abiotic stressors are jeopardizing them from attaining their complete genetic potential, consequently affecting world crop production (Gill et al., 2011; Senthil-Nathan, 2013). The increasing mean global temperature rise is creating a negative impact on the production of horticultural crops (Singh et al., 2016; Senthil-Nathan, 2015). The mineral nutrient status of plants serve as critical factors that determines plant growth, vigour besides crop yield and these nutrients play a particular role in contributing to the survival of crop plants under environmental stress conditions (Hassan et al., 2008). Meant for healthy besides vigorous development, it is essential for plants to take up large quantities of major nutrients (nitrogen, potassium and phosphorus) and minor amounts of micro nutrients (iron, nickel, chromium, manganese, zinc, boron, copper and molybdenum) from the soil (George et al., 2008).

Global fertilizer production has reached an overwhelming quantity of 207.98 MT annually. This increase is attributed to the application of higher amounts of fertilizer per unit area (23kg/0.23 ha) (FAOSTAT, 2014). Further complications have arisen with the disproportionate increase of fertilizer application – grain land ratio, over the years. This has subsequently not only up surged the production costs, but also has degraded the environment to the extent that the soils are becoming unfit for agriculture. As a paradox, these economic expenses did not go in par with the income or yield, thus increasing the financial burden of farmers worldwide (Sunarpi et al., 2010). And so, the determinations to capitalize on the plant nutrient absorption by means of natural products that comprehends all plant nutrients, is a premeditated approach to subdue the applications of inorganic fertilizers (Naguib, 2011). These

Abbreviations: CA, Chaetomorpha antennina; LSE, Liquid Seaweed Extract; EDS, Energy Dispersive Spectroscopy; EDAX, Energy Dispersive Analysis X-Ray; SFS, Starter fertilizer solution; CF, Chemical fertilizer; MGT, Mean Germination time; GP, Germination Percentage; GE, Germination Energy; SVI, Seedling vigour index; MC, moisture content; *chl a*, Chlorophyll a; *chl b*, Chlorophyll b; TSS, Total Dissolved Solids

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organic fertilizers either obtained from animal or plant source, encompasses all essential components, which can enhance the plant yield and soil fertility (Mathur et al., 2015).

Marine ecosystem serves as bioactive compounds' repository comprising of sulfated polysaccharides, terpenoids, phenolics, lactones, sterol and fatty acids, possessing pharmacological, along with plant growth stimulating properties (Chanthini et al., 2012). Seaweeds, as rich sources of secondary metabolites, are reported with plant biostimulant potentials by various research activities (Battacharyya et al., 2015; Godlewska et al., 2016; Layek et al., 2018). Also the seaweed extracts are applied towards the enhancement of seed germination, to confer resistance in plants against biotic-abiotic stressors besides, intensifying the soil nutrient uptake. These positive outcomes are credited to the presence of phytohormones and other plant growth promoting substances (Jannin et al., 2013). The seaweed products have also entered the global bio-fertilizer markets.

Tomato (*Lycopersicon esculentum* Mill), is a leading vegetable crop, in terms of gloal cultivation and consumption. The global tomato production has increased (from 3 to 17 million tonnes from 2009 to 2016). Conversely, the total tomato cultivation area is subsiding (FAO, 2018) with a reported 19 million dollar loss due to the effect of several abiotic stressors (IPCC, 2014). Keeping these aspects, the current study was carried out to investigate the biostimulant potential of *C. antennina* in terms of seed germination, growth, nutritional and yield parameters of *L. esculentum*.

2. Materials and methods

2.1. Seaweed collection and identification

The seaweed, *C. antennina* (Cladophoraceae), were collected from the rocks of the coastal regions of Colachel beach (Fig. 1), Kanyakumari (8°14′ 5168″ N and 77° 14' 35.209" E), Southern Tamil Nadu, India, during low tide period, August (2018). The collected seaweeds were washed in seawater several times to remove impurities, sand particles along with epiphytes, brought to the research laboratory in taped up polythene bags. The seaweed was washed thoroughly in tap water many times, wearied and spread on blotting paper to remove excess water. It was then shade dried for 3 h.

2.1.1. Preparation of C. anteniina - Liquid seaweed extract (CA-LSE)

The washed seaweeds were then severed into minor fragments, boiled in distilled water (100 gms/1 L) for 1 h. It was then filtered through cheese cloth (double layered) and stored in 4 °C in refrigerator until further use. The test concentrations of LSE were prepared by diluting the extract with distilled water (CA-LSE 20, 40, 60, 80 and 100%).

2.1.2. X-ray–Energy dispersive spectroscopic (EDS) analysis of C. antennina

The cell wall elemental composition of seaweed was performed by Energy Dispersive Analysis X-Ray - EDAX (BRUKER).

2.2. Preparation of fertilizer solutions

Ammonium dihydrogen phosphate (Merck) was used as starter fertilizer solution (SFS) by mixing 1 mg of $NH_4H_2PO_4$ in sterile distilled water (10 ml), designated as positive control for seed priming assays. For the purpose of analysing yield and quality parameter assays, a mixture of urea, triple super phosphate and muriate of potash (8:8:8) were used, designated as Chemical fertilizer (CF).

2.3. Seed collection

Tomato seeds, PKM1, were purchased from Tamil Nadu Agricultural University (TNAU), Coimbatore. Seeds of undeviating dimensions and hue were carefully chosen for study, surface sterilised with 0.1% mercuric chloride, washed thrice in sterile distilled water and the experimentation was carried out at the Sri Paramakalyani Centre for Excellence in Environmental Science, Manonmaniam Sundaranar University, Alwarkurichi, from July to August 2018.

2.3.1. Seed preparation

Germination assays were performed using tomato seeds (5seeds/plate), primed with 10 ml of respective treatment solutions for 12 h, in sealed conical flasks. . The seeds were then removed, spread in a filter paper to blot out the solutions at room temperature (24 h). Then it was placed in pre-labelled sterile petri dishes (9 cm) over filter paper (Whatman No. 5), that was moistened (sterile distilled water), instantaneously taped up with parafilm (Merck) to prevent moisture loss, incubated (25 ± 2 °C/alternative16:8 h LD). The plates were checked for radicle protrusion (> 2 mm) on a daily routine (hint of germination). Ten seeds were tested for each concentration of LSE.

To investigate possible effects of CA-LSEs on tomato plant's vegetative growth and yield, small pot field experiments, containing sterilised soil, were conducted by sowing the primed seeds. The pots were labelled based on the treatments. CF was used as positive control for greenhouse assay.

2.4. Biostimulant assays

2.4.1. Germination assays

Germination was recorded every day by counting the emerging hypocotyls. The mean germination time (MGT) was premeditated (Ellis and Roberts 1980), by counts made on the time taken for 1, 10, 25, 50, 75 and 100% of the seeds to germinate and expressed as days.

$$MGT = \frac{\Sigma\ (n\ T)}{\Sigma\ n}$$

where.n = number of newly germinated seeds at time T (25 °C).T = hours from the beginning of the germination test.Σ n = final germination.

Fig. 1. Sample collection site – *C. antennina* attached to rocks.

*100% will refer to the total number of seeds germinated after exposure to highest LSE concentration.

The germination percentage (GP) was calculated using the following formula

$$\text{Germination percentage (GP)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Seed Germination Energy (GE) was calculated according to the formula

$$\text{Germination Energy} = \frac{\text{Number of germinating seeds}}{\text{No. of total seeds per test post germination for 3 days}} \times 100$$

Seedling vigour index (SVI) was calculated (Orchard, 1977) by the following formula:

$$\text{SVI} = \text{Seedling length (cm)} \times \text{germination \%}$$

2.4.2. Seed weight (dry and wet)

The biomass (wet and dry weight in mg) of the seeds primed with CA-LSEs and SFS solutions for 24 h were determined with the help of an electronic balance after oven-drying at 40 °C for two days.

2.4.3. Seed imbibition

Seed imbibition was determined by measuring the weight of seeds (100 seeds/treatment) before and during priming with SFS and LSEs at 6, 12, 24, 36 and 48 h and plotting the water imbibition curve by determining the seed moisture content (MC) and through means of which, the seed imbibition time was calculated (Larreta et al., 2008). Seeds primed in distilled water served as control. The MC was calculated by the formula

$$\text{Moisture content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Dry weight}} \times 100$$

2.4.4. Root and shoot length

Total plant height, root-shoot lengths and ratio were measured post 20 days by uprooting the plants from the pots. Total plant height, leaf and branch number per plant, along with root-shoot dry matter (oven drying at 70 °C) weights (g) were determined about 65 days post priming.

2.5. Yield and quality parameter assays

The effect of CA – LSE on yield of tomato plants were estimated by the fruit weight after 65 days of seed priming. The quality parameters of treated tomato plants were calculated in terms of chlorophyll (a & b), carotenoids (Nagata and Yamashita 1992), total soluble solids (TSS) (Hedge et al., 1962), ascorbic acid (Horwitz et al., 1970) and phenol (Sadasivam, 1992) contents.

2.6. Statistical analysis

All the tests were repeated five times. The effect of LSE on seeds were determined by analysis of variance, one-way (ANOVA), and the treatment means were compared by Tukey-family error test ($P < 0.05$) by using Minitab®17 software package.

3. Results

3.1. Seaweed identification

The seaweeds collected was identified as *C. antennina* (Fig. 1), based on their organoleptic features (Table 1) and microscopic examination (Nikon H600L, Japan; 40X), that displayed un-branched structure, with a thin cell wall and box shaped cells that encompassed mucilaginous thin walled single layer meristoderm (Fig. 2).

Table 1
C. antennina - Organoleptic features.

Traits	*C. antennina*
Habitat	Marine
Shape	Filamentous
Size	7 cm
Colour	Light green
Odour	Fishy
Taste	Salty
Base	Holdfast
Texture	Smooth

3.1.1. EDX analysis of C. antennina

The elemental composition of the seaweed elucidated via EDX analysis (Fig. 3), revealed the presence of six compounds on seaweed cell surface, oxygen, Na, Mg, S, Cl and Ca. Oxygen was present in higher quantities (69.08%), followed by chlorine (10.06%), Calcium (6.75%), Magnesium (6%), Sodium (5.51%) and Sulphur (7.79).

3.2. Bio-stimulant assays

3.2.1. Seed bioassay

The tomato seeds in control began to germinate on the third day (Fig. 4), at 86.54 h ($F_{4,\ 20} = 30.33$; $P < 0.0001$), reaching 50% emergence at 119.8 h ($F_{6,\ 28} = 53.23$; $P < 0.0001$) and 163.2 h for 100% emergence ($F_{4,\ 20} = 30.33$; $P < 0.0001$). The untreated seeds were recorded with a GE of 10% ($F_{6,\ 28} = 41.7$; $P < 0.005$) and SVI of 457.18 ($F_{6,\ 28} = 42.41$; $P < 0.005$). The seeds treated with SFS germinated at 85.54 h ($F_{4,\ 20} = 30.33$; $P < 0.0001$), with 4.8 days MGT ($F_{6,\ 28} = 53.23$; $P < 0.0001$) (Fig. 5) and 148.8 h for 100% emergence ($F_{4,\ 20} = 38.16$; $P < 0.0001$), with a GE of 20% ($F_{6,\ 28} = 41.7$; $P < 0.005$) in addition to 743.75 SVI ($F_{6,\ 28} = 42.41$; $P < 0.005$).

Primed seeds germinated earlier besides, increasing their germination percentage than that of untreated (Fig. 6). CA-LSE treated seeds germinated on the second day, taking 46.8 (60%), 40.6 (80%) and 40 (100%) hours with respective MGT of 3, 2.58 and 2.3 days ($F_{6,\ 28} = 53.23$; $P < 0.0001$). Consequently, CA-LSE treated seeds also reached 100% emergence within 3.62(80%) ($F_{4,\ 20} = 59.7$; P 0.0001) and 3.2 days (100%) ($F_{4,\ 20} = 63.31$; $P < 0.0001$). CA-LSE treated seeds (80 and 100%) also displayed corresponding higher levels of SVI (Table 2) and GE of 1271.31, 1300.4 ($F_{6,\ 28} = 42.41$; $P < 0.005$) and 97.6, 98.8 ($F_{6,\ 28} = 41.7$; $P < 0.005$) respectively (Fig. 7).

3.2.2. Seed imbibition

The weights of tomato seeds were determined after priming with distilled water (control), SFS and CA-LSEs, in terms of wet and dry weight (mg). Control seeds weighed 0.113 mg ($F_{6,\ 28} = 49.3$; $P < 0.003$) 48 h after priming and 0.013 mg dry weight ($F_{6,\ 28} = 37.1$; $P < 0.005$). SFS treated seeds weighed 0.1396 mg ($F_{6,\ 28} = 49.3$; $P < 0.003$) post priming and 0.0218 mg ($F_{6,\ 28} = 37.1$; $P < 0.005$) dry weight (Table 2). The CA-LSE treated seeds weighed a wet weight of 0.143, 0.15, 0.2,0.305 and 0.39 mg ($F_{6,\ 28} = 49.3$; $P < 0.003$) at 20, 40, 60, 80 and 100% respectively; displaying corresponding dry weights of 0.0286, 0.0314, 0.04, 0.0527 and 0.057 mg ($F_{6,\ 28} = 37.1$; $P < 0.005$).

Subsequent moisture content of seeds increased with time and treatment doses. Control seeds' imbibition reached 9.9% after 48 h ($F_{4,\ 20} = 43.03$; $P < 0.0001$), which was lesser than that of SFS treated seeds, 10.1% ($F_{4,\ 20} = 60.15$; $P < 0.005$), which was near to the seed imbibition percentage of seeds treated with lower concentrations of CA-LSEs (20 and 40%), *viz.*, 10.8 ($F_{4,\ 20} = 42.7$; $P < 0.0001$) and 11.5% ($F_{4,\ 20} = 50.01$; $P < 0.002$). Comparatively higher imbibition % of

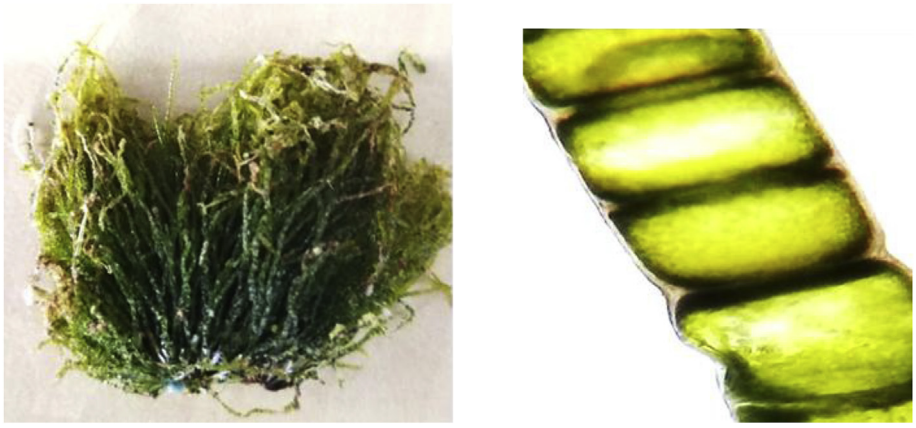

Fig. 2. Macroscopic and microscopic images of *C. antennina.*

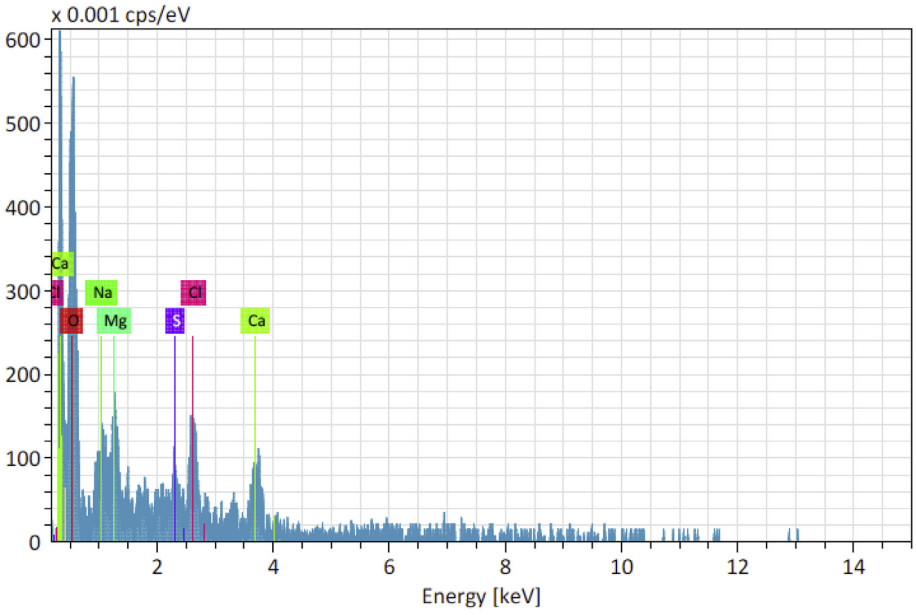

Fig. 3. SEM-EDX - energy dispersive spectrum of *C. antennina.*

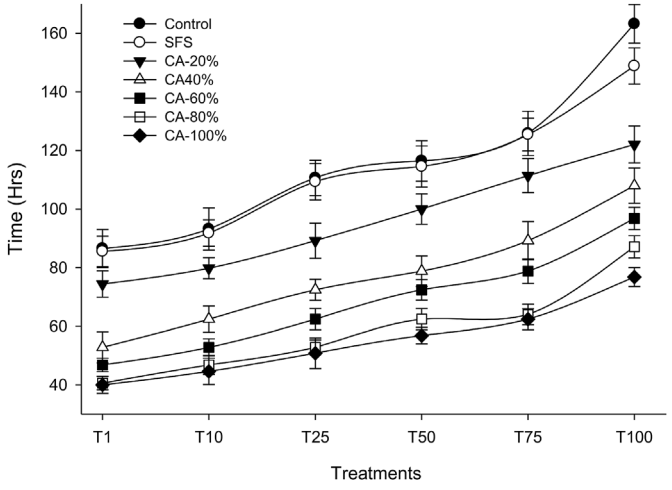

Fig. 4. Germination time course of CA-LSEs primed seeds. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference ($P < 0.05$) in a Tukey's test.

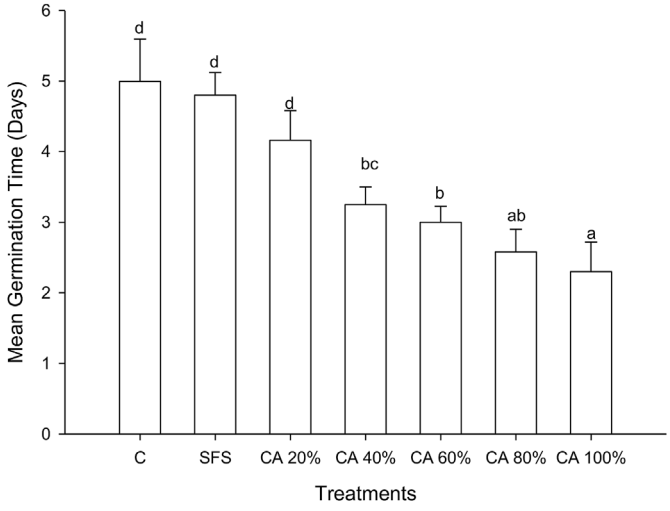

Fig. 5. MGT of CA-LSEs primed seeds. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference ($P < 0.05$) in a Tukey's test.

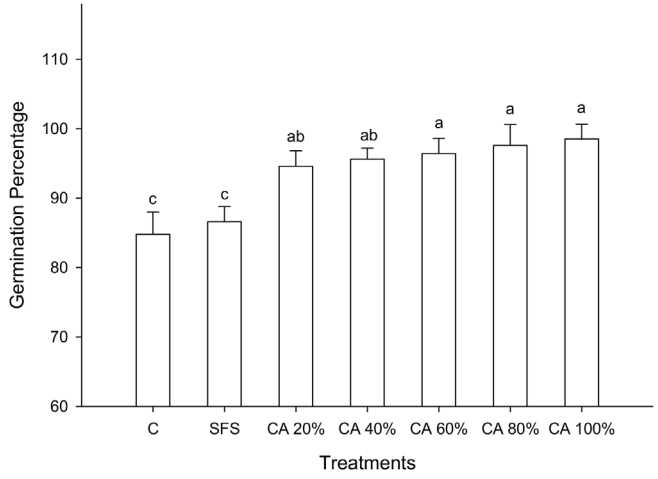

Fig. 6. Germination Percentage of CA-LSE primed seeds. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference (P < 0.05) in a Tukey's test.

Table 2
Effect of CA-LSEs on SVI and seed biomass.

Treatments	Seedling Vigour Index	Seed weight (mg)	
		Wet weight	Dry weight
C	457.18 ± 2.76^{g}	0.113 ± 0.007^{g}	0.013 ± 0.007^{g}
SFS	743.75 ± 3.02^{f}	0.1396 ± 0.003^{f}	0.0218 ± 0.003^{f}
CA 20%	1044.04 ± 3.01^{e}	0.143 ± 0.005^{e}	0.0286 ± 0.001^{e}
CA 40%	1058.24 ± 2.45^{d}	0.150 ± 0.003^{d}	0.0314 ± 0.003^{d}
CA 60%	1233.96 ± 1.43^{c}	0.2 ± 0.003^{c}	0.040 ± 0.003^{c}
CA 80%	1271.31 ± 2.65^{b}	0.305 ± 0.002^{b}	0.0527 ± 0.002^{b}
CA 100%	1300.4 ± 2.51^{a}	0.39 ± 0.002^{a}	0.057 ± 0.003^{a}

*Columns denoted by a different letter are significantly different at P ≤ 0.05 in a Tukey's test.

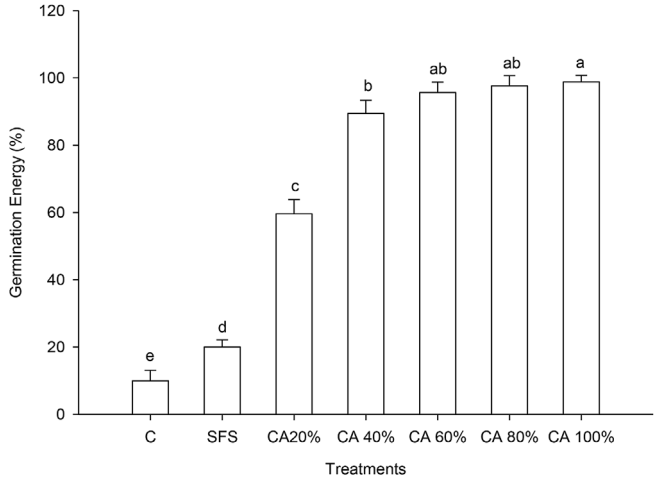

Fig. 7. Germination energy of seeds primed with CA-LSEs. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference (P < 0.05) in a Tukey's test.

11.7 ($F_{4,\ 20}$ = 39.5; P < 0.0001), 11.8 ($F_{4,\ 20}$ = 42.05; P 0.005) and 12% ($F_{4,\ 20}$ = 38.52; P < 0.0001) were observed in higher treatment dosages (60, 80 and 100%) (Fig. 8).

3.2.3. Growth parameter assay

The tomato seedlings reached a height of 16 cm at the end of 20 days ($F_{6,\ 28}$ = 40.45; P < 0.003), 6 cm shoot ($F_{5,\ 24}$ = 62.40;

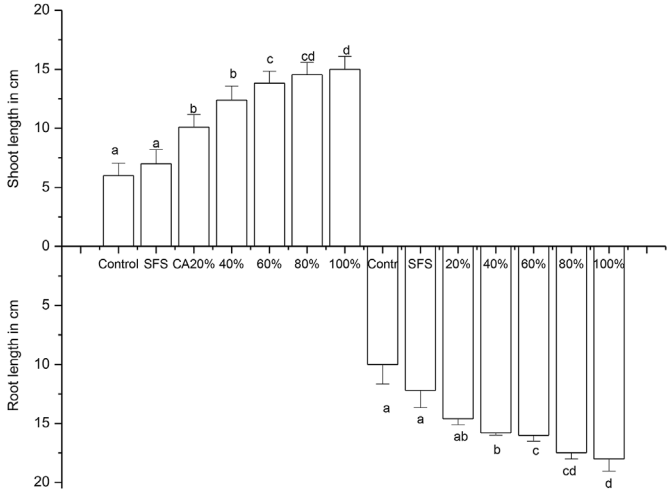

Fig. 8. Effect of CA-LSEs on seed imbibition capabilities of tomato seeds. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference (P < 0.05) in a Tukey's test.

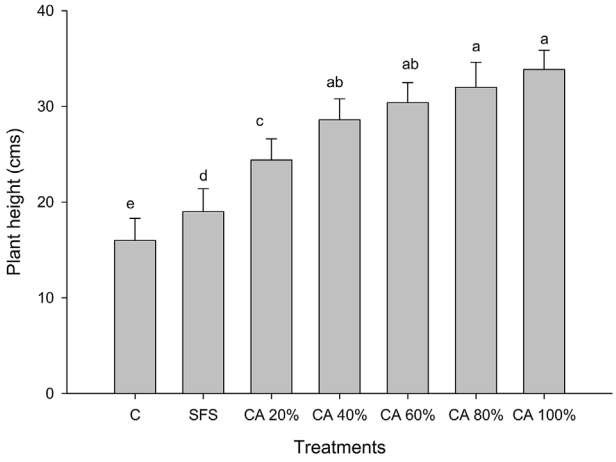

Fig. 9. Root and shoot lengths of plants emerged out of CA-LSE primed seeds. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference (P < 0.05) in a Tukey's test.

P < 0.005) and 10 cm root lengths ($F_{6,\ 28}$ = 56.3; P < 0.005) (Fig. 9). The SFS treated seeds displayed a total plant height of 19 cm ($F_{6,\ 28}$ = 40.45; P < 0.003), with shoot-root lengths of 7 cm ($F_{5,\ 24}$ = 62.40; P < 0.005) and 12.2 cm ($F_{6,\ 28}$ = 56.3; P < 0.005) respectively. CA – LSE stimulated plant growth even at lower concentrations, displaying 24.4, 28.6 and 30.38 cm ($F_{6,28}$ = 40.45; P < 0.003) plant height (Fig. 10), with respective shoot-root lengths of 10.1, 12.38 and 13.82 cm ($F_{5,24}$ = 62.40; P < 0.005) and 14.6, 15.8 and 16 cm ($F_{6,\ 28}$ = 56.3; P < 0.005). At higher treatment concentrations (80 and 100%), the plant height increased to 32 and 33.86 cm ($F_{6,28}$ = 40.45; P < 0.003) with shoot-root lengths of 14.54, 15 cm ($F_{5,24}$ = 62.40; P < 0.005) and 17.48, 18 cm ($F_{6,28}$ = 56.3; P < 0.005) respectively.

The root shoot ratios increased from 0.68 to 0.82 in control to SFS treated seeds (Fig. 11), reaching a maximum of 1.69, 1.7 and 1.86 at 60, 80 and 100% CA-LSE treated seeds ($F_{6,28}$ = 58.7; P < 0.000). Seeds treated with CA-LSE 20, 40 and 60% displayed root shoot ratios of 0.9, 1.38 and 1.69 respectively ($F_{6,28}$ = 58.7; P < 0.000).

3.2.4. Yield assays

The tomato seeds, that were not treated, gave rise to plants with 115.39 cm height ($F_{6,28}$ = 36.4; P < 0.005), comprising 10.5 branches ($F_{6,\ 28}$ = 57.02; P < 0.005) and 67 leaves ($F_{6,28}$ = 60.18; P < 0.004);

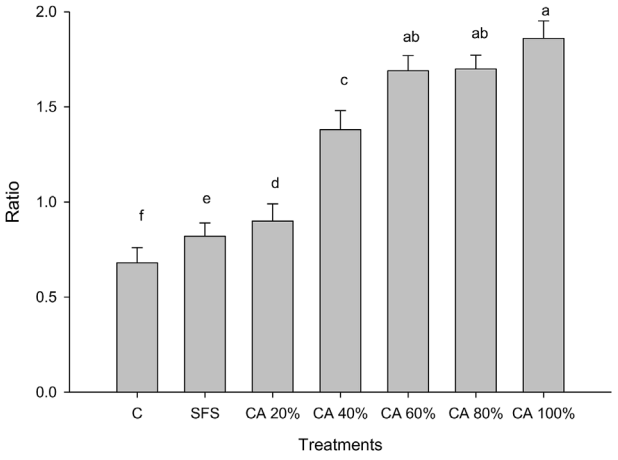

Fig. 10. Effect of CA-LSEs seed priming on plant height. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference (P < 0.05) in a Tukey's test.

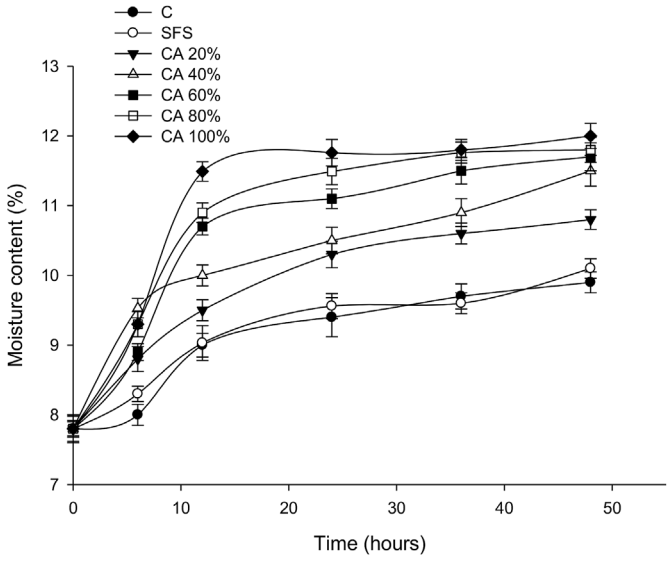

Fig. 11. Effect of CA-LSEs seed priming on root-shoot ratio of emerged plants. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference (P < 0.05) in a Tukey's test.

total root-shoot dry matters of 7.04 ($F_{6,\ 28}$ = 38.9; P < 0.0001) and 72 g ($F_{6,\ 28}$ = 49.7; P < 0.001), that yielded 1.7 kg ($F_{6,28}$ = 44.72; P < 0.003) tomatoes (Table 3). Seeds primed with CF grew up to 118 cm height ($F_{6,28}$ = 36.4; P < 0.005), with 13 branches ($F_{6,28}$ = 57.02; P < 0.005) – 70 leaves ($F_{6,28}$ = 60.18; P < 0.004), 7.1 ($F_{6,28}$ = 38.9; P < 0.0001) and 86 g ($F_{6,28}$ = 49.7; P < 0.001) root-

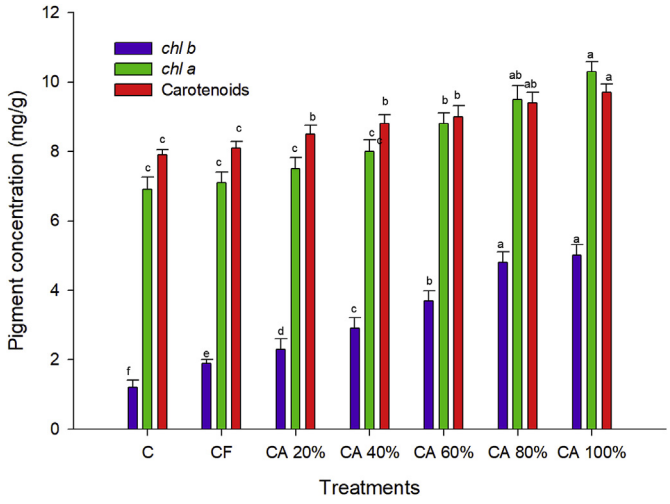

Fig. 12. Effect of CA-LSEs on pigment concentration of leaves. Mean (± SEM) followed by the same letter in individual larval instar in bars indicate no significant difference (P < 0.05) in a Tukey's test.

shoot dry weights, yielding 2.1 kg tomatoes ($F_{6,28}$ = 44.72; P < 0.003). The CA-LSE primed seeds gave rise to plants with increased height, 120, 123, 126, 129 and 135 cm ($F_{6,28}$ = 36.4; P < 0.005), displaying 15, 17.9, 18.5, 20.7 and 22.51 branches ($F_{6,28}$ = 57.02; P < 0.005) along with 72, 77, 82, 89 and 95 leaves ($F_{6,28}$ = 60.18; P < 0.004), weighing dry weight biomass of 7.59, 8.3, 8.89, 9.86 and 10.86 g root ($F_{6,28}$ = 38.9; P < 0.0001) and 93, 96, 112, 118 and 125.57 g shoot ($F_{6,28}$ = 49.7; P < 0.001), yielding 2.4, 2.8, 3.1, 3.7 and 4.01 kg tomatoes ($F_{6,28}$ = 44.72; P < 0.003) with increase in treatment doses *viz.,* CA-LSE 20, 40, 60, 80 and 100%.

3.2.5. Quality parameter assays

The leaves of control plants displayed pigment contents of 6.9 mg/g of *chl a* ($F_{6,\ 28}$ = 68.06; P < 0.005), 1.2 mg/g of *chl b* ($F_{6,28}$ = 32.6; P < 0.005) and 7.9 mg/g of carotenoid ($F_{6,28}$ = 22.81; P < 0.003). The *chl a*, *chl b* and carotenoid contents of CF treated seed-plant were estimated to be 7.1 mg/g ($F_{6,28}$ = 68.06; P < 0.005), 1.9 mg/g of ($F_{6,28}$ = 32.6; P < 0.005) and 8.1 mg/g ($F_{6,28}$ = 22.81; P < 0.003) and that of CA-LSE primed seed-plants were 7.5, 8, 8.8, 9.5 and 10.3 mg/g ($F_{6,28}$ = 68.06; P < 0.005), 2.3, 2.9, 3.7, 4.8 and 5.01 mg/g ($F_{6,28}$ = 32.6; P < 0.005) and 8.5, 8.8, 9, 9.4 and 9.7 mg/g ($F_{6,28}$ = 22.81; P < 0.003), that increased with increase in treatment concentrations of CA-LSE 20, 40, 60, 80 and 100% respectively (Fig. 12).

The fruit quality assays of control and CF treated plants displayed 4.7and 4.85 Brix TSS ($F_{6,28}$ = 41.53; P < 0.005), 90 and 93 mg/100 g FM ascorbic acid ($F_{6,28}$ = 59.14; P < 0.005) and 7.33 and 9.02 mg GAE /100 g of phenol ($F_{6,28}$ = 58.41; P < 0.003). The CA-LSE treated plant fruits of 20, 40, 60, 80 and 100% concentrations were found to

Table 3
Growth and yield of tomato influenced by the seed priming effect of CA-LSEs.

Treatments	Plant height (cm)	No. of branches/plant	No. of leaves/plant	Dry matter weight (g /plant)		Yield (kg/plant)
				Root	Shoot	
C	115.93 ± 2.17^{e}	10.5 ± 1.8^{f}	67.8 ± 1.28^{g}	7.04 ± 0.76^{d}	72 ± 2.3^{g}	1.7 ± 0.57^{c}
CF	118.41 ± 1.2^{d}	13.06 ± 1.9^{e}	70.6 ± 1.36^{f}	7.1 ± 0.8^{d}	86 ± 2.01^{f}	2.1 ± 0.51^{c}
CA 20%	120.28 ± 1.13^{c}	15.4 ± 1.38^{d}	72 ± 1.03^{e}	7.59 ± 0.64^{d}	93 ± 1.9^{e}	2.4 ± 0.59^{c}
CA 40%	123.5 ± 1.82^{bc}	17.9 ± 1.7^{c}	77 ± 1.64^{d}	8.3 ± 0.74^{c}	96 ± 2.7^{d}	2.8 ± 0.4^{bc}
CA 60%	126.09 ± 2.05^{a}	18.5 ± 1.54^{b}	82 ± 1.9^{c}	8.89 ± 0.8^{c}	112 ± 2.14^{c}	3.1 ± 0.42^{b}
CA 80%	129.56 ± 1.36^{a}	20.7 ± 1.52^{a}	89 ± 1.64^{b}	9.86 ± 0.61^{b}	118 ± 2.75^{b}	3.7 ± 0.43^{a}
CA 100%	135.16 ± 1.86^{a}	22.1 ± 1.04^{a}	95 ± 1.27^{a}	10.6 ± 0.71^{a}	125.7 ± 2.53^{a}	4.01 ± 0.49^{a}

*Columns denoted by a different letter are significantly different at P ≤ 0.05 in a Tukey's test.

Table 4
Effect of CA-LSEs on biochemical constituents of tomato fruit.

Treatments	TSS (Brix)	Ascorbic acid (mg/100 g FM)	Phenol (mg GAE /100 g)
C	4.7 ± 0.08^{e}	90 ± 1.2^{g}	7.33 ± 1.2^{f}
CF	4.85 ± 0.05^{d}	93 ± 1.32^{f}	9.02 ± 1.0^{e}
CA 20%	5.01 ± 0.032^{c}	97 ± 1.47^{e}	9.98 ± 0.96^{de}
CA 40%	5.05 ± 0.024^{b}	110 ± 1.51^{d}	10.17 ± 0.5^{de}
CA 60%	5.073 ± 0.035^{ab}	115 ± 1.7^{c}	11.08 ± 0.43^{c}
CA 80%	5.097 ± 0.014^{a}	120 ± 1.32^{b}	11.75 ± 0.8^{b}
CA 100%	5.1 ± 0.012^{a}	125 ± 1.4^{a}	12.8 ± 0.736^{a}

*Columns denoted by a different letter are significantly different at $P \leq 0.05$ in a Tukey's test.

contain 5.01, 5.05, 5.073, 5.097 and 5.1 Brix TSS ($F_{6,28} = 41.53$; $P < 0.005$), 97, 110, 115, 120 and 125 mg/100 g FM ascorbic acid ($F_{6,28} = 59.14$; $P < 0.005$) as well as 9.98, 10.17, 11.08, 11.75 and 12.8 mg GAE /100 g of phenol ($F_{6,28} = 58.41$; $P < 0.003$) respectively (Table 4).

4. Discussion

Seed pre-treatment is regarded a major strategy in sustainable agricultural practices. This technique has a direct influence on the physiology of crop plants, in ways in-numerable, thereby positively affecting crop development and yield (Lanka et al., 2017). In this study, the tomato seed priming effects of CA-LSEs were evaluated in terms of emergence, plant growth and yield. The results of the series of experiments revealed that the CA-LSEs altered the physiology of tomato seed in ways such that they offer as potential candidates as plant growth biostimulants. The most reliable response of CA-LSE treated seeds were attributed to the early emergence of primed seeds, emerging 2 days earlier than untreated seeds. Additionally, the treated seeds displayed higher germination percentage (98.52%), besides amplified germination energy (98.8%), which were 88% higher compared with control. Prompting of plant germination besides seedling growth by priming with LSEs of different seaweeds has been reported in several crops. The extracts of green seaweed, *Codium tomentosum* induced the germination percentages of pepper seeds (68%) and aubergine seeds (91%) (Demir et al., 2006). Likely, seaweed suspensions, *Ascophyllum nodosum* and *Laminaria hyperborea* increased the germination percentage of barley, by increasing oxygen availability to the embryo (Moller and Smith, 1999).

The vigour of seedlings emerged out of primed seeds were three times better to that of untreated. The potentials of seaweed extracts to augment the SVI was also proved in seeds of brinjal, tomato and chilli, primed with LSEs of *U. lactuca, U. reticulata, Padina pavonica* and *S. johnstonii* (Patel et al., 2018). The seed imbibition rate also increased as a result of seed treatments with CA-LSE by 2.1% in comparison with control. The weight of seed is directly proportional to the plant establishment capacity, thereby forming an ecologically crucial character in plant progress (Kalaivani et al., 2016). The weight of primed seeds increased with time, thus yielding in plants of higher biomass and productivity (Poveda et al., 2003; Kalaivani et al., 2018). This was also stated by Wulff (1986), who studied the seed mass-yield relationship in *Desmodium paniculatum*. The higher seed biomass of CA-LSE treated seeds were also in agreement with the results of Karthikeyan (2016), who reported the biomass increase of red gram (*Cajanus cajan*) and peanut (*Arachis hypogaea*)seeds primed with *Kappaphycus alvarezii* extracts. Further, *Codium decorticatum* extracts were also able to increase the biomass of *Capsicum annum* seeds (Vijayakumar et al., 2018).

The growth parameter assay performed in terms of plant height, root-shoot lengths and ratio proved the bio-stimulant capacity of CA-LSE, displaying altered root shoot development, displaying an increase in the total plant height to 135 from 115.93 cm. These plants also appeared to have increased number of branches and leaves, subsequently yielding three times that control plants. This was in agreement with the results published earlier by Kavipriya et al. (2011), who studied the effect of green seaweed extracts of *Ulva lactuca* and *Caulerpa scalpelliformis* on green gram seeds. The extracts were found to increase the seed germination as well as the growth parameters. Similar results were also observed in red gram treated with the extracts of *Sargassum myriocystum*, that displayed enhanced emergence speed (18%), seedling length (15%), Fresh and dry weight (11%) and vigour index, leaf area (13%) when compared with control (Amabika, 2015).

Besides increasing the vegetative growth of the tomatoes, the CA-LSEs were also found to increase their yield. This was also in agreement with the previous works which deals with increased yield reports of tea treated with LSEs of *Hypnea musciformis*, *Laurencia robtusa*, *Padina tetrastromatica* and *Stoechospermum mariginatum* (Thevanathan and Bhavan, 2005); pea and black gram treated with LSEs of *Ulva lacuta, Turbinaria conoides* and *Sargassum polycystum* (Ramamoorthy et al., 2006a, b; 2007). Additionally, the yield potencies of tomato, pulses, *Triticum aestivum* were found to be stimulated by the application of LSEs of *Kappaphycus alvarezii* (Zodape et al., 2008), *Pandina pavonia* (Bai et al., 2011) and *S. wightii* (Kumar and Sahoo, 2011).

The biostimulant mechanism of CA-LSE is chiefly owed to the copiousness of plant essential nutrients and phytohormones present in the seaweeds (Spinelli et al., 2010). These components play an imperative part in augmentation of cell size besides cell division, in addition to root-shoot development that is favoured by the cumulative effects of auxins and cytokinins. As an added credit, the micronutrients in the LSE serve as soil conditioners, thereby improving the soil health (Liu, 2011). Organic fertilizers have been reported to reduce nitrate leaching by enhancing the activities of soil denitrifying bacteria, increasing the bioavailability of nitrogen to plants (Kramer et al., 2006).

There was a linear increase in these biochemical contents in the leaves (Chlorophyll a & b and carotenoids) and fruits (TSS, Ascorbic acid and phenols) of plants emerged out of primed seeds. Leaf greenness increase might be facilitated by the nutrients supplied by the extracts. An increase in chlorophyll and carotenoid levels enhance light capture potential of leaves, thereby increasing the rate of photosynthesis (Gross et al., 1991). TSS determines the flavour of tomato fruits that is influenced by the intake of nitrogen, calcium, potassium, sulphur and copper (YARA, 2019). Ascorbic acid concentration is greatly subjective by the environment influences (Davey et al., 2007). Ascorbic acid levels determine the nutritional quotient of tomato fruit, apart from involving in pathways of plant adaptation during stress conditions; also serving as antioxidants (Smirnoff N, 2000). Phenols are important secondary metabolites produced by plants that play a major role in defense against disease and pest (Prasad BD et al., 2017). A likely increase in these biochemical parameters were also reported in tomato plants treated with LSE of *Sargassum* sp. (Kumari et al., 2011) and *U. fasciata, S. ilicifolium* and *Gracilaria corticata* treated *Trigonella foenum - graecum*. The extract of red seaweed, *S. swartziiwith* was reported to enhance the nutritional quality of cowpea (*Vigna unguiculata* L. Walp) by improving their phytochemical content (Vasantharaja et al., 2019).

The seaweed was subjected to EDX analysis, in order to elucidate their elemental composition. The analysis revealed the presence of six elements (O, Na, Mg, S, Cl and Ca). Several elements, macro and micro nutrients are essential for plant growth, among which oxygen is regarded as a major structural nutrient, which is abundant in the seaweed cell wall (69.08%). Oxygen forms the basis for carbohydrates, which provide the strength of cell walls, stems, and leaves, and are also sources of energy for the plant and organisms that consume the plant. Additionally the presence of Sodium (Na), a functional plant nutrient (5.51%), that promotes maximal biomass yield of a plant (Subbarao et al., 2003). The secondary nutrients, Calcium (Ca), Magnesium (Mg) and Sulphur (S) are required by crops in relatively large amounts. Ca promotes plant growth, while Mg, being an essential chlorophyll component is critical for photosynthesis apart from regulation of other

nutrient uptake. Sulphur, is also essential for protein synthesis, chloroplast growth and function, a vital part of electron transport chain and also nitrogen fixation (Mengel et al., 2001). Although the LSE lack major plant nutrients such as N, P and K, these micro nutrients enhance the nutrient and water uptake of plants. Additionally, they also improve the soil conditions for the proliferation of plant growth promoting biota in the rhizosphere. These micro biotas metabolize the inorganic NPK to organic form, thereby making the rhizosphere rich in essential plant nutrients that are readily available for assimilation (Yakhin et al., 2017; Raghunandan et al., 2019). Layek et al. (2019) reported relative reduction in soil nutrient content with increase in application doses of sap of seaweeds *K. alvarezii* and *Gracilaria edulis* on maize. This was due to effect of seaweed sap on the enhancement of nutrient uptake capabilities of maize.

The application of LSE from the seaweed, *C. antennina* as seed priming agent displayed optimistic outcome on the improvement of vegetative growth and yield of tomato plant. Moreover, the CA-LSEs also escalated the plant and fruit biochemical components, which may increase their resistance against various biotic and abiotic stressors, which is the major challenge faced by agriculturalists round the globe, who aim for sustainable food production.. Under these circumstances, utilisation of seaweeds for agricultural application is economical as they are a renewable marine source, easy to culture and also no specialised methods are required for the LSE preparation or formulation for application. Coupled with their benefits of potential biostimulant as well as soil conditioner agents, seaweeds can be regarded as virtuous catalyst for the betterment of sustainable agricultural food production.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101190.

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