

## Influence of seaweed liquid fertilizer from *Ulva lactuca* Linnaeus, 1753 on seed germination and growth parameters of Green gram

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Seaweed extracts are extensively used as fertilizers across the globe. However, their mechanism of action and effects are less understood and need to be studied in detail. The present study investigated the effect of *Ulva lactuca* seaweed extract as a liquid fertilizer (SLF) on the growth characteristics of green gram. The seeds of green gram were soaked in varied concentrations of aqueous seaweed extract like 2, 4, 6, 8 and 10 %. Additionally, the SLF was applied on germinated seedlings as foliar spray treatment in 2 days interval. The maximum growth parameters were observed in foliar spray treatment at 8 % concentration and in seed soaking treatment at 2 % concentration. The seaweed extract was effective in influencing the growth characteristics of green gram.

[**Keywords:** Foliar spray, Green gram, Growth parameters, Seaweed liquid fertilizer, Seed soaking, *Ulva lactuca*]

### Introduction

Seaweed metabolites are becoming a preferable source of natural organic fertilizers. Their nutritious content promotes faster seed germination and improves crop yield and disease resistance. Most chemical fertilizers are non-biodegradable and recalcitrant in nature. However, natural seaweed derivatives are biodegradable, non-polluting, non-toxic and non-hazardous to any life<sup>1</sup>. Seaweed biofertilizers can be a viable alternative to less desirable chemical fertilizers. Algal components can be an effective fertilizing input for agricultural crops due to their high level of organic matter, micro and macro elements, vitamins, fatty acids and rich growth-promoting regulators<sup>2</sup>. Seaweeds contain all the necessary plant essential minerals like Cu, PO<sub>4</sub>, Mn, Ca, K, Mg, etc. Recently, seaweed fertilizers have gained global attraction and are more successful in markets than chemical fertilizers. Many macroscopic marine seaweed types are used as compost manures in Indian coastal areas. Apart from being used as farm yard manure, their usage as a liquid fertilizer is becoming much more attractive for foliar spraying to induce faster crop growth and yield in cereal crops, horticultural plants and orchards<sup>3</sup>.

*Ulva lactuca* Linnaeus, 1753 is a ubiquitous marine macro algae. It is commonly known as a Sea lettuce, as it resembles lettuce in morphology. Their green thallus anchors them to shells, rocks and other substratum. *Ulva lactuca* can be found in natural

waters under eutrophic conditions<sup>4</sup>. Free floating thallus ranges in colour between light green and dark green, transparent and olive green spots<sup>5</sup>. The colour ranges of *U. lactuca* is related to the nitrogen content. The more intensively green-coloured thallus represents a higher level of nitrogen contents.

### Materials and Methods

#### Seaweed collection

The marine green seaweed *U. lactuca* was collected in June 2018 from the Mandapam coastal region of Gulf of Mannar (latitude 09°16'14" N; longitude 79°07'10" E) located along the southeast coast of Ramanathapuram district, Tamil Nadu. The seaweeds were collected in clean, sterile containers after rinsing in seawater to remove unwanted impurities or adhering particles. Thereafter, the seaweeds were thoroughly washed in tap water to remove any salt deposits on the algal surface. Post washing, the samples were shade dried at room temperature for seven days. The dried seaweed samples were pulverized with the help of a mixer grinder and preserved for future use in clean, sterile, airtight containers.

#### Phytochemical analysis of seaweed

To 500 ml of aqueous ethanol prepared in a conical flask by combining 100 ml distilled water and 400 ml absolute ethanol added 50 g of fine algal powder. The

mixture was incubated at room temperature in dark for 2 days in a shaker. The suspension was filtered using Whatman No. 1 filter paper and the filtrate was condensed by rotary evaporator at 40 °C. The condensed hydro-ethanolic crude extract was stored at 4 °C for future use. Phytochemical tests were conducted to determine metabolites contained in the macroalga *U. lactuca*<sup>6</sup>.

#### **Saponin test**

A 100 µl of crude extract diluted in 5 ml of distilled water was heated, cooled and filtered. The filtrate transferred to a test tube was made up to 10 ml with distilled water and shaken vigorously for 10 seconds and observed for a formation of stable persistent froth.

#### **Flavonoids test**

Flavonoid screening is done by placing 1 ml of the crude extract in a watch glass. The solution was evaporated to dryness under vacuum rotary evaporator, and the remains were moistened with acetone followed by addition of fine powder of boric acid and oxalic acid. The mixture was carefully heated over a water bath, avoiding overheating. Finally, 1 ml of ether was added to the residue and mixed well. The watch glass with its content were placed in a UV Transilluminator. A yellow fluorescence with UV exposure at 366 nm confirmed the presence of flavonoids in the sample.

#### **Triterpenoid and steroids test: Liebermann-Burchard reaction**

The 2 ml of crude extract was evaporated to dryness and the residue was taken in a porcelain dish. The residue was dissolved with 0.5 ml each of chloroform and acetic acid anhydride. 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was then added through the tube wall. The presence of triterpenoids is characterized by the formation of a brownish or violet ring at the boundary of the solution. While, a blue-green ring formation indicates the presence of steroids.

#### **Essential oils test**

The presence of essential oil is characterized by a distinctive odour generated by the residue obtained after evaporating 1 ml of the crude extract in a porcelain dish.

#### **Alkaloids test**

A 2 ml of hydro-ethanolic crude extract was made into a residue by evaporating it in a porcelain cup. The residue was further mixed with 5 ml of 2N HCl. Three drops of Dragendorff's reagent and 3 drops of Mayer reagents were added to portions of the resulting solution

separately, whereby a formation of orange precipitate in the former tube and yellow precipitate in the latter tube indicates alkaloid presence.

#### **Tannin test**

One millilitre of the extract was reacted with a 10 % of iron (III) chloride solution. A dark blue or greenish-black colour formation indicates a positive result.

#### **Glycosides test**

In a test tube, 0.1 ml of extract was evaporated over a water bath, and the left contents were dissolved by 5 ml of acetic acid anhydride. To this, 10 drops of concentrated sulfuric acid were added. A blue or green product formation indicates glycosides presence.

#### **Preparation of seaweed liquid fertilizer**

A 100 g of powdered seaweed was added to 1000 ml of distilled water and the contents were heated for 45 min at 60 °C. Four layered muslin cloth was used for filtering after cooling the contents. A standard crude – 10 % (w/v) aqueous seaweed extract was prepared from which five different concentrations 2, 4, 6, 8 and 10 % (w/v) were prepared using double distilled water.

#### **Experimental design and treatments**

In the pot culture experiment, two major treatment setups - 1 for algal extract soaked seeds germination cum growth and the other for unsoaked seeds germination cum algal extract foliar spray supplemented growth were maintained for the study. In each treatment, different concentrations of algal extract served as sub-treatments for soaking or spraying purposes.

#### **Soil sample collection and processing**

Six soil samples were collected in the month of May from close proximity to the magnesite mining area in Salem, Tamil Nadu. The soil from the sites was reported to be deficient in nitrogen, phosphorus and potassium due to the leaching and mining activities<sup>7</sup>. The soils were slightly alkaline with a high C/N ratio of 30. Soil samples were pooled and processed following the methods of Thilagam & Hemalatha<sup>8</sup>. Soil lumps were broken down, removed foreign materials like roots or pebbles and were left at room temperature for a day to dry. When sufficiently dried, the soil was passed through a 2 mm sieve. Polystyrene pots of 10 × 10 × 10 cm dimensions to hold 1 kg of sieved soil was used. Co 6 variety green gram crop *Vigna radiata*, also known as *Phaseolus aureus* Roxb. was chosen for the pot culture experiment.

#### **Soaking, sowing, germination of seeds & foliar spray**

The Seaweed Liquid Fertilizer (SLF) was prepared with different concentrations *i.e.*, 2, 4, 6, 8 and 10 %. Seeds of green gram variety Co 6 were procured from commercial markets in Salem. The seeds of homogenous size and appearance were selected for the study. Surface of the seeds was disinfected with 5 % aqueous sodium hypochlorite for 2 min and then rinsed thrice in sterile distilled water. The test seeds were soaked in prepared concentration of SLF and water control for 12 h. Five seeds were sown per pot for each treatment. Experiments were carried out in triplicates, making a total of 15 nos. per treatment. The whole setup was duplicated (one for seed soaking & the other for foliar spray treatment). The soaked and without soaking seeds, constituting 2 primary treatment setups, were sown in the treatment pots accordingly. The latter was used for a foliar treatment study. Treatment pots were arranged in a Completely Randomized Design (CRD) in five treatment replicates of each two primary setups, under controlled conditions of 27 °C, 15 h photoperiod and 55 % relative humidity in a glasshouse. Pots were watered uniformly every day. Then the seeds were observed for germination percentage and seedling growth. The germination percentage was calculated by dividing the number of germinated seeds by the total number of test seeds and multiplying by 100.

From the 5<sup>th</sup> day of sowing, when most of the sown seeds in pots had germinated, in the seed soaking treatment pots, 100 ml of distilled water was sprinkled over seedlings at regular intervals of 5 days. Similar to this, the SLF foliar treatment was also commenced in the other experimental pot setup. In each of the SLF foliar treatments, 100 ml of appropriate aqueous seaweed extract was applied using a sprinkler. Thereafter, spraying was followed at intervals of 5 days in each treatment for the next 15 days. A growth period of 15 days was allowed.

#### **Measurement of plant growth characteristics**

The growth parameters were noted after allowing growth at uniform environmental conditions. The germinated seeds were allowed to grow for 15 days, and the plant growth characteristics were observed by taking six representative seedling samples per treatment group. The shoot and root length were recorded in centimetres using a scale from above and below the seedling's root collar centre. Shoot length was measured almost every day. The other parameters, including root length, plant fresh/dry

weight and chlorophyll contents<sup>9</sup>, were recorded on 15<sup>th</sup> day of the pot experiment in all treatments. Drying was carried out at 105 °C for half an hour in porcelain crucibles in a muffle furnace to record dry weight. Average parameter values were calculated per representative sample in all treatments.

#### **Estimation of chlorophyll**

In this current study, the chlorophyll estimation of the green gram seedlings was performed. The spectrophotometric method<sup>10</sup> was used for the estimation of chlorophyll contents. The fresh 5 g sample was weighed and put in an acetone–water mixture (8 ml, 90 % v/v acetone) to extract the pigments. After vigorous shaking and 10 min ultrasonication, the tube was left undisturbed for 48 h in the dark at 4 °C. Acetone was then added at room temperature to compensate for any evaporation. Later, the solution was centrifuged for 5 min at 7,000 rpm (Universal 320R, Hettich make). Collected the supernatant and obtained spectrophotometric readings (optical density) at 665, 645 and 630 nm. The following equations were used to calculate the concentrations of chlorophyll content:

$$Chl-a = 11.6 \times OD\ 665 - 1.31 \times OD\ 645 - 0.14 \times OD\ 630^{(ref.\ 11)}$$

$$Chl-b = 20.7 \times OD\ 645 - 4.34 \times OD\ 665 - 4.42 \times OD\ 630^{(ref.\ 12)}$$

$$Chl-c = 55.0 \times OD\ 630 - 4.64 \times OD\ 665 - 16.3 \times OD\ 645^{(ref.\ 13)}$$

Where, *Chl-a*, *Chl-b* and *Chl-c* are the concentrations (mg.l<sup>-1</sup>) of chlorophyll-*a*, *b*, and *c*, respectively, and *OD xxx* is the optical density measured at the specified wavelength. The optical density measurements were made in quartz cuvettes of 1 cm light path. If the absorbance value exceeded 0.8 units, the sample was diluted with the solvent (acetone–water) to bring the measurement within range. The elemental analysis was performed in atomic emission spectroscopy after the digestion process as described by Ertani *et al.*<sup>14</sup>.

#### **Results and Discussion**

The phytochemical properties of *Ulva lactuca* have been analyzed in the present study. Phytochemical compounds of *U. lactuca* included tannins, flavonoids, steroids and glycosides (Table 1). The steroids were found in greater amounts than other phytochemical

Table 1 — Phytochemical analysis of *Ulva lactuca*

S. No.	Phytochemical compounds	Results
1.	Flavonoid	+
2.	Triterpenoid	+
3.	Steroid	+
4.	Essential oil	-
5.	Alkaloid	-
6.	Tannin	+
7.	Glycosides	+

(‘+’ Presence; ‘-’ Absence)

compounds. Similar is the case with triterpenoids. Steroids were mostly found as steroid alcohol. Plant phytoosterols, namely sitosterol, stigmasterol, kaempferol, etc., are used in drug manufacturing<sup>15</sup>. Similar results were also observed for biochemical components such as phenol, flavonoid, alkaloids and tannin of *U. lactuca*<sup>3</sup>. The nitrogen, phosphorous, potash and organic matter, as well as the presence of other trace elements, improve the biological values of seaweed as fertilizer<sup>16</sup>. The analysis of *U. lactuca* seaweed extract revealed that amongst the macronutrients, the values of total nitrogen were maximum, followed by potassium, sulphur, magnesium, calcium and phosphorous similar to the previous study of Sridhar & Rengasamy<sup>17</sup>. An elemental analysis of the *Ulva* extract revealed the content of nitrogen (86.5 mg/l), potassium (43 mg/l), sulphur (22 mg/l), magnesium (6.7 mg/l), calcium (2 mg/l), phosphorous (2.29 mg/l) and also showed the presence of Fe and Cl micronutrients.

The application of seaweed extract has been shown to enhance the moisture holding capacity of the soil. Abiotic stresses like drought, salinity, nutrient deficiency, leaching and high temperature reduces the crop yield to much extent. Application of Seaweed Liquid Fertilizer (SLF) helps the plant to overcome such stresses<sup>18</sup>. Further, the seaweed metabolites present in the SLF were found to be beneficial in reducing diseases by stimulating the defence enzymes. A stimulating effect of *U. lactuca* SLF on both germination and growth of green gram (*Vigna radiata*) plants was seen in the current study. Seeds soaked in 2 % concentration of SLF showed higher rates of germination compared with other concentrations like 4, 6, 8 and 10 % (Fig. 1). This is in agreement with the previously reported study that seaweed extract induce variations in seed germination rate<sup>19</sup>. It can be inferred that more than the macronutrients supplied in the extract, the inhibitory substances of the extract, namely tannins or alkaloids, may negatively influence seed germination. The interactions between a seed and the adjacent soil

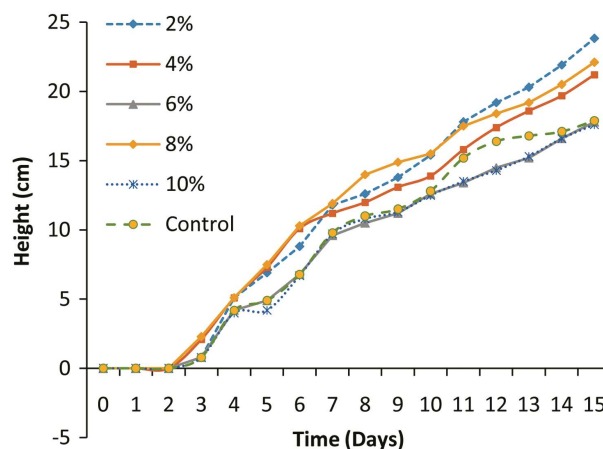


Fig. 1 — SLF influence on the shoot length of green gram plants in seed soaking treatments

microbes may be quite necessary for its successful germination. Germination may be affected when such interactions are disturbed by interference from external factors or chemical compounds. The presence of tannins and alkaloids at higher concentrations in the extract must have hindered the seed–soil microbe interaction that helps in germination. It becomes evident that the supply of any growth factor or plant growth-supporting nutrients is of little use in the germination process. A better germination percentage when compared to control may be due to the influence of extract upon suppression of seed vigour by altering the environment around the seed.

The SLF also influenced the growth characteristics of plant such as shoot length and root length. Compared to control and other soaking treatments, seeds soaked at 2 % extract had high values of growth parameters like root length ( $8.0 \pm 0.5$  cm), dry weight ( $107 \pm 0.78$  mg) and shoot length ( $23 \pm 0.83$  cm). The performance was even better than their foliar spray counterpart, which received foliar treatment with 2 % algal extract. In the foliar treatment batch, an increase in performance was noted with an increase in foliar spray concentration up to 8 %. In foliar spray treatments, a comparatively higher value of growth parameters were observed in 8 % concentration (Fig. 2 and Table 2), with a shoot length of  $20 \pm 0.32$  cm and root length of  $8.6 \pm 0.56$  cm. This foliar concentration also showed the highest fresh weight ( $342 \pm 2.15$  mg) and dry weight ( $117 \pm 1.93$  mg). A decreased growth performance at a higher concentration of 10 % foliar spray may be due to the increase in phytotoxic components of extracts. They can exhibit direct influence or may indirectly

affect plant-beneficial microbes in phyllosphere or rhizosphere. The nutrients, minerals and other growth factors in *Ulva lactuca* extract supported the foliar treatment seedlings, as evidenced in the results showing good incremental growth with increased extract concentration up to 8 %.

In this study, the observed plant growth promotion up to 8 % algal extract (foliar spray) must be due to good

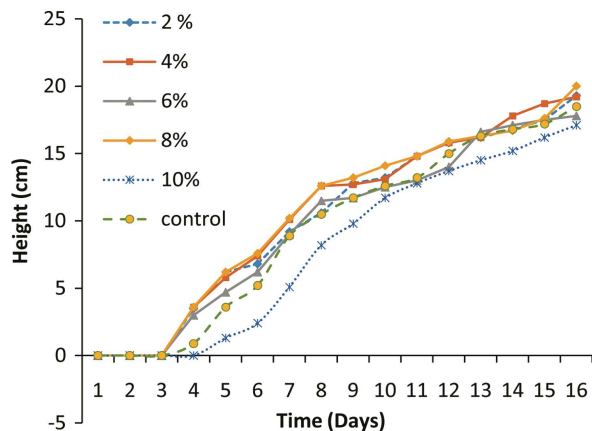


Fig. 2 — SLF influence on the shoot length of green gram plants in foliar spray treatment

root development and nutrition absorption from adjacent and deep soil area as discussed in earlier studies by Zodape *et al.*<sup>20</sup> and Siddharthan *et al.*<sup>21</sup>. In earlier studies, SLF had influenced growth parameters like number of leaves & flowers, number of fruits and biomass of *Abelmoschus esculentus* (L.) plants<sup>22</sup>. The present investigation showed increased chlorophyll content in algal extract-treated green gram seedlings when compared to controls without extract treatments, where the results did coincide with an earlier report<sup>23</sup>. Whapham *et al.*<sup>24</sup> also reported that the chlorophyll content of cucumber and tomato plants had been raised by *Ascophyllum nodosum* SLF application. Foliar applications of SLF had improved chlorophyll production in rice crop<sup>25</sup>. Similarly, seaweed extract prepared from *U. lactuca* and *Padina tetrastromatica* were found to have chlorophyll synthesis and fertilizing ability. In the present study, high chlorophyll content was obtained in the treatment of 2 % extract-soaked seeds ( $11.93 \pm 0.21 \mu\text{g l}^{-1}$ ) and 8 % foliar spray ( $15.53 \pm 0.3 \mu\text{g l}^{-1}$ ) treatment (Tables 3 & 4), compared with other treatments and control. Among above two, the 8 % foliar spray-treated seedlings showed higher chlorophyll contents along with enhanced other growth

Table 2 — Effect of seed soaking and foliar spray with SLF on green gram fresh weight, dry weight and root length at 15<sup>th</sup> day of experimentation

SLF concentration	Seed soaking			Foliar spray		
	Fresh weight (mg)	Dry weight (mg)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)	Root length (cm)
2 %	315±2.63	107±0.78	8±0.5	302±2.16	99±2.46	6.2±0.3
4 %	306±2.01	103±1.23	7±1.02	315±2.85	104±1.59	6.6±0.8
6 %	274±1.79	105±1.47	7.5±0.9	323±1.98	111±1.27	7.1±0.42
8 %	304±1.46	92±1.56	6.5±0.5	342±2.15	117±1.93	8.6±0.56
10 %	262±2.09	89±2.03	6±0.4	297±1.05	96±2.85	5.9±1.04
Control	300±3.06	101±1.74	6.2±0.7	301±2.06	99±1.73	6.1±0.56

Table 3 — Effect of SLF seed soaking on green gram chlorophyll content at 15<sup>th</sup> day of experimentation

SLF concentration	Chlorophyll-a ( $\mu\text{g l}^{-1}$ )	Chlorophyll-b ( $\mu\text{g l}^{-1}$ )	Chlorophyll-c ( $\mu\text{g l}^{-1}$ )	Total chlorophyll ( $\mu\text{g l}^{-1}$ )
2 %	88.7±14	33.1±63	52.6±74	119.3±21
4 %	84.26±42	29.1±14	43.21±18	108.6±24
6 %	76.32±23	23.6±32	42.1±15	101±12
8 %	65.6±16	21.7±32	42.6±47	79.9±16
10 %	53.21±13	12.1±1	30.14±18	71.81±32
Control	63.8±32	18.14±23	63.21±17	79.71±29

Table 4 — Effect of SLF foliar spray on green gram chlorophyll content at 15<sup>th</sup> day of experimentation

SLF concentration	Chlorophyll-a ( $\mu\text{g l}^{-1}$ )	Chlorophyll-b ( $\mu\text{g l}^{-1}$ )	Chlorophyll-c ( $\mu\text{g l}^{-1}$ )	Total chlorophyll ( $\mu\text{g l}^{-1}$ )
2 %	43.587±5	33.446±48	41.73±35	78.6±30
4 %	72.6±15	43.25±74	44.1±42	94.7±14
6 %	52.1±95	24.1±69	42.7±93	72.4±12
8 %	102.5±7	53.6±74	77.46±58	155.3±40
10 %	52.6±24	42.5±31	46.96±17	45.8±25
Control	63.5±35	19.87±41	65.29±15	81.5±30

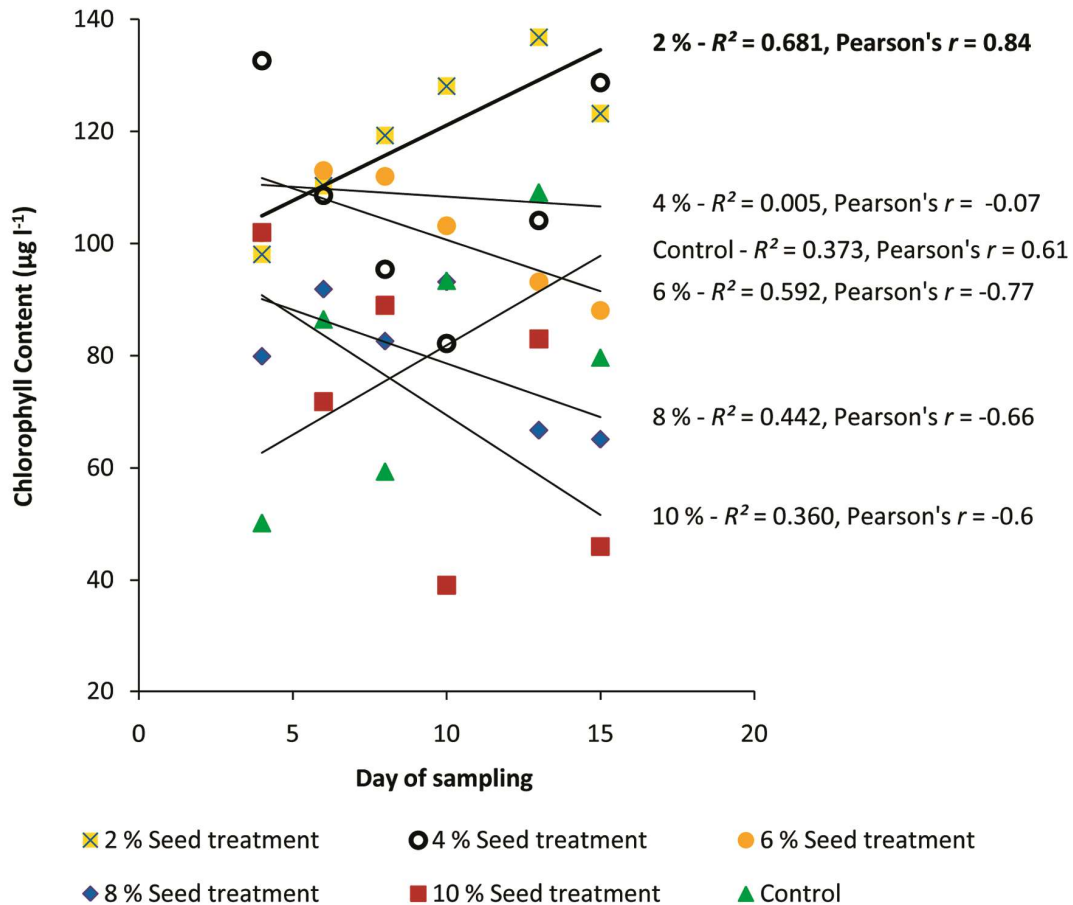


Fig. 3 — SLF Seed soaking influence on the Chlorophyll content of growing green gram plants

characteristics. The increase in chlorophyll content in the foliar treatment was 90.5 % when compared to the control, but the seed soaking treatment exhibited 49.5 % increase in chlorophyll content than in its control treatment. An increase in chlorophyll content should support photosynthesis to supply energy for the seedling growth.

A high chlorophyll-*a* content was obtained in 8 % foliar spray treatment followed by 2 % extract-soaked treatment during the 15<sup>th</sup> day of study. A correlation study with the chlorophyll contents checked at regular intervals against different extract concentrations which showed a high positive correlation (Fig. 3) with 2 % extract seed treatment ( $r = 0.84$ ) and with 8 % extract foliar treatment ( $r = 0.82$ ; Fig. 4). In seed treatment with higher extract concentrations, a negative correlation was observed.

The present study shows that for optimal plant growth, the foliar spray of *Ulva lactuca* algal extract is much beneficial when applied at regular intervals

and to some extent by one-time treatment of seeds before sowing but only at lower concentrations. The study also infer that algal phytochemicals have a combination of bioactive compounds exhibiting both phytotoxicity and plant growth enhancers. The seeds have sufficient stored embryonic nutrients during early stages. There is a requirement for nutrient and growth factors only at later growth stages for optimum plant growth. Some algal phytochemicals may either directly plant toxic or indirectly affect the plant-associated microbiomes which in turn, affect growth. The study suggests that the biomantle including microbes around the seed exerts a profound effect whether positive or negative on plant growth. A combination of seed soaking and foliar spray treatment are further scopes of this study. The study shows that crop growth can be enhanced in low-nutrient areas like magnesite mine adjacent soils when proper irrigation and organic biostimulant treatments are combined favourably.

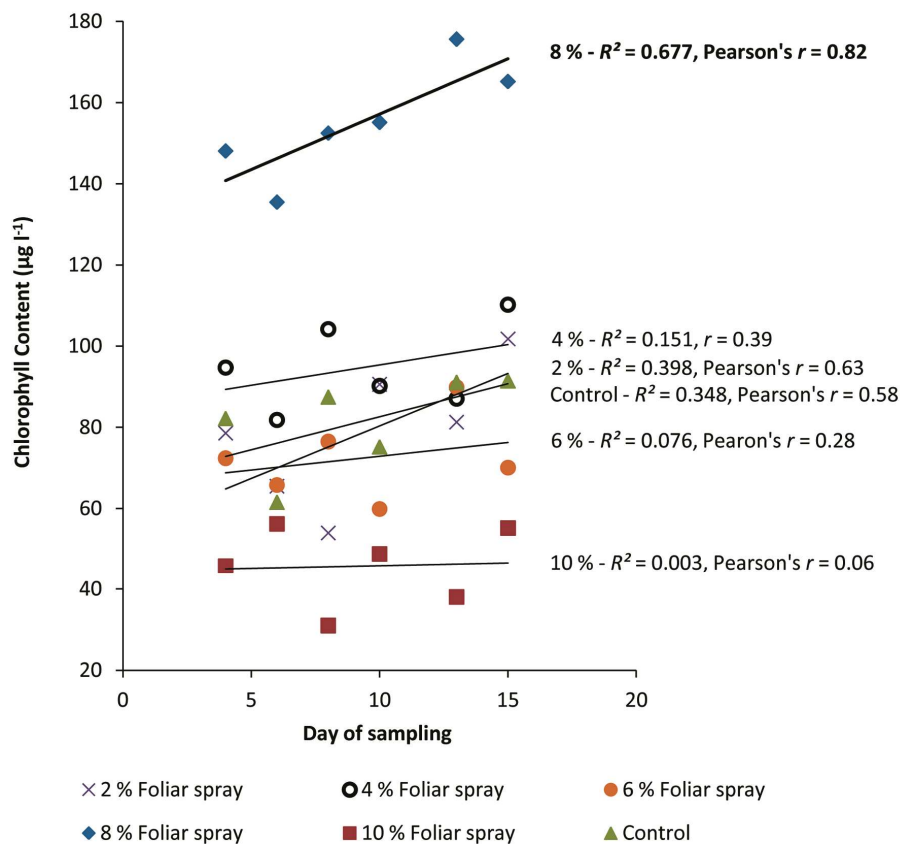


Fig. 4 — SLF foliar spray influence on the Chlorophyll content of growing green gram plants

### Conclusion

The present study concluded that *Ulva lactuca* seaweed liquid fertilizer was very effective in acting as a growth promoter of green gram plants when applied as foliar spray or as seed coating. Results clearly demonstrate that *U. lactuca* seaweed liquid fertilizer is a promising agent for enhancement and productivity of the representative test plant, and hence, the same can be inferred for other related agricultural crops. The seaweed-extracted metabolites are good organic biostimulants for crops and can be adopted for organic farming and soil fertility improvement.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

### Ethical Statement

This is to certify that the reported work in the paper entitled “Influence of seaweed liquid fertilizer from *Ulva lactuca* Linnaeus, 1753 on seed germination and growth parameters of Green gram” submitted for publication is an original one and has not been submitted for publication elsewhere. We further certify that proper citations to the previously reported work have been given and no data/table/figure has been quoted verbatim from other publications without giving due acknowledgement and without the permission of the author(s). The consent of all the authors of this paper has been obtained for submitting the paper to the Indian Journal of Geo-Marine Sciences.

### Author Contributions

NH conceptualized the work, supervised and contributed for manuscript editing and finalizing. GK

carried out the sample collection and experimentation and prepared the original draft. NS, GV & SK contributed to analyzing SPSS software Ver.14 and did formal analysis.

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